2014-1690, -1164

United States Court Of Appeals for the Federal Circuit

SYNTRIX BIOSYSTEMS, INC.,

Plaintiff/Cross-Appellant,

v.

ILLUMINA, INC.,

Defendant/Appellant,

APPEALS FROM THE U.S. DISTRICT COURT FOR THE WESTERN DISTRICT OF WASHINGTON
IN CASE NO. 10-CV-05870-BHS, JUDGE BENJAMIN H. SETTLE

ILLUMINA'S OPENING BRIEF

Juanita R. Brooks
Craig E. Countryman
Fish & Richardson P.C.
12390 El Camino Real
San Diego, CA 92130

Adam Shartzer
Fish & Richardson P.C.
1425 K Street, NW, 11th Floor
Washington, DC 20005

March 12, 2014

CERTIFICATE OF INTEREST

Counsel for Illumina, Inc., certifies the following:

1. The full name of every party represented by me is: Illumina, Inc.

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is: N/A.

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party represented by me are: Baillie Gilford & Co.

4. The names of all law firms and the partners or associates that appeared for the party now represented by me in the trial court or are expected to appear in this court are:

Fish & Richardson P.C.: Juanita R. Brooks; Craig E. Countryman; Adam Shartzer;

Osborn Maledon, PA: David B. Rosenbaum; Eric M. Fraser; Brett L. Dunkelman; Erick S. Ottoson;

Graham & Dunn: Diane Meyers; David C. Lundsgaard.

Dated: March 12, 2014

/s/ Craig E. Countryman
Craig E. Countryman

TABLE OF CONTENTS

Page

Certificate of Interest i

Statement of Related Cases vi

Statement of Jurisdiction vii

Statement of the Issues viii

Introduction 1

Statement of the Facts 2

I. The Technology: Genetic Testing by Attaching Probe DNA to Surfaces. 2

II. The '682 Patent: Using a Porous Coating With Multiple Layers of Small Particles to Increase Surface Area for Attaching Probes. 5

A. The Perceived Problem: A Signal Was Difficult to Detect from Prior Art Arrays Without Using Expensive Equipment. 5

B. The Proposed Solution: A "Porous Coating" With Small Particles and Multiple Layers to Increase Signal Strength. 6

C. The Claims: A Porous Coating Comprising a "Three-Dimensional Network of Particles." 11

III. Illumina's Technology: Using a Single Layer of Large Beads-in-Wells to Ease Manufacturing, While Developing Better Scanners to Read Them. 12

IV. This Lawsuit: Syntrix Stretches Its Patent Beyond What it Describes. 15

A. Claim Construction and Other Pre-Trial Proceedings. 15

TABLE OF CONTENTS (continued)

Page

B. The Trial 16

C. The Verdict, and the District Court's Post-Trial Rulings. 19

Summary of the Argument 21

Argument 22

I. The Claims Are Invalid Because the Specification Does Not Describe a Large-Particle Porous Coating Like Those Syntrix Says They Cover. 22

A. Precedent Prohibits a Patentee from Claiming a Class of Structures that Are Not Described in the Specification 22

B. The '682 Patent Does Not Describe a Large-Particle Coating Like Those Covered by the Claims As Construed. 25

C. Syntrix Failed to Identify Any Description of Large-Particle Coatings to Support its Claims. 28

II. The Judgment Should Be Reversed or Vacated Based on the Proper Treatment of the "Three-Dimensional Network" of Particles Limitation. 31

A. The Court Should Correct the Claim Construction and Either Enter Judgment of Non-Infringement, or, at a Minimum, Vacate. 31

B. The Claims Are Invalid Under the District Court's Construction Because the Patent Never Describes a Single-Layer Coating. 41

III. If Liability is Affirmed, the Court Should Vacate the Damages Award 43

Conclusion 47

TABLE OF AUTHORITIES

Page(s)

Cases

Anascape, Ltd. v. Nintendo of Am. Inc.,
601 F.3d 1333 (Fed. Cir. 2010) ..... *passim*

Atlantic Research Marketing Sys., Inc. v. Troy,
659 F.3d 1345 (Fed. Cir. 2011) 28

Bayer CropScience AG v. Dow AgroSciences LLC,
728 F.3d 1324 (Fed. Cir. 2013) 36

Centocor Ortho Biotech, Inc. v. Abbott Labs.,
636 F.3d 1341 (Fed. Cir. 2011) 22

Cordis Corp. v. Boston Scientific Corp.,
658 F. 3d 1347 (Fed. Cir. 2011) 40, 41

Gentry Gallery, Inc. v. Berkline Corp.,
134 F.3d 1473 (Fed. Cir. 1998) ..... *passim*

Hologic, Inc. v. SenoRx, Inc.,
639 F.3d 1329 (Fed. Cir. 2011) 34

ICU Medical, Inc. v. Alaris Medical Systems, Inc.,
558 F.3d 1368 (Fed. Cir. 2009) 24, 26, 42

LaserDynamics, Inc. v. Quanta Computer, Inc.,
694 F.3d 51 (Fed. Cir. 2012) 46

Liebel-Flarsheim Co. v. Medrad, Inc.,
481 F.3d 1371 (Fed. Cir. 2007) 28

Lighting Ballast Control LLC v. Philips Elecs. N. Am. Corp.,
2014 WL 667499 (Fed. Cir. Feb. 21, 2014) (*en banc*) 32

Lizardtech, Inc. v. Earth Resource Mapping, Inc.,
424 F.3d 1336 (Fed. Cir. 2005) 23, 24, 28, 35

Lucent Techs., Inc. v. Gateway, Inc.,
580 F. 3d 1301 (Fed. Cir. 2009) 43, 45, 46

TABLE OF AUTHORITIES (continued)

Page(s)

Philips v. AWH Corp.,
415 F.3d 1303 (Fed. Cir. 2005) (*en banc*) 32, 34, 39

ResQNet.com v. Lansa, Inc.,
594 F.3d 860 (Fed. Cir. 2009) 45

Retractable Tech. v. Becton, Dickson, & Co.,
653 F.3d 1296 (Fed. Cir. 2011) 34, 36

Tronzo v. Biomet, Inc.,
156 F.3d 1154 (Fed. Cir. 1998) ..... *passim*

Uniloc USA, Inc. v. Microsoft Corp.,
632 F.3d 1292 (Fed. Cir. 2011) 43, 46

Other Authorities

FED. R. EVID. 402 43

FED. R. EVID. 702 43

STATEMENT OF RELATED CASES

There have been no prior appeals related to this case before this or any other appellate court. No case in this or any court will directly affect or be directly affected by this Court's decision in the pending appeals.

STATEMENT OF JURISDICTION

The district court had jurisdiction over this patent infringement case under 28 U.S.C. §§ 1331 and 1338, and it exercised jurisdiction over Syntrix's now-dismissed state law claims under 28 U.S.C. § 1367.

The district court entered an amended judgment on July 1, 2013, (A1-2), and denied Illumina's post-trial motions under Rules 50 and 59 on November 4, 2013, thereby resolving all remaining issues in the case. (A32-41.) Illumina filed its Notice of Appeal on December 3, 2013, (A209-10), within 30 days of the order denying the post-trial motions, making it timely under Federal Rule of Appellate Procedure 4(a)(1) and 4(a)(4)(A). Syntrix filed its Notice of Cross-Appeal on December 16, 2013, (A212-13), within 14 days of Illumina's Notice, which makes it timely under Federal Rule of Appellate Procedure 4(a)(3).

This Court thus has jurisdiction over the appeal and cross-appeal under 28 U.S.C. § 1295(a)(1).

STATEMENT OF THE ISSUES

1. Whether claims that require a "porous coating" comprising a "gelled network of particles" lack written description where:

    a. The stated purpose of the invention requires a porous coating with many layers of small particles;

    b. The patent never mentions a large-particle porous coating and instead describes only small-particle coatings with particle sizes of at most 2,000 angstroms;

    c. The patent teaches that particles bigger than 1,000 angstroms are too large "to be useful in the present invention"; but

    d. Syntrix asserts that the claims cover a structure with "large," 31,000 angstrom particles.

2. Whether the judgment should be set aside where:

    a. The claims require a porous coating comprising a "gelled network of particles" that is "three-dimensional network of particles";

    b. The district court erroneously declined to construe this term in accordance with its ordinary meaning, which is a porous coating made up of multiple layers of particles;

    c. Under the correct construction, Illumina would not infringe as a matter of law because its products have, at most, a single layer of particles; and

    d. Under the district court's construction, the claims are invalid for lack of written description because the specification never describes a porous coating with a single layer and instead suggests that the results from a single layer would be "insufficient."

3. If the Court affirms on liability, whether the $115 million judgment should be vacated where the district court erroneously admitted testimony encouraging the jury to double the royalty rate based on a stock transfer that was unrelated to compensation for any patent rights.

INTRODUCTION

This appeal seeks to correct a $115 million judgment that Syntrix obtained by stretching a narrow patent on a particular way of solving a particular problem to sweep in Illumina's wholly-different solution to a wholly-different problem.

Syntrix's '682 patent-in-suit sought to strengthen the optical signals emitted from arrays of DNA ligands attached to a substrate. The '682 patent criticizes prior art arrays as having insufficient surface area for attaching ligands, which generated a weak signal and thus required costly scanners to analyze. The '682 patent's solution was to apply a "porous coating" made of a "three-dimensional network of particles" to the substrate and attach the ligands throughout the porous coating. The patent emphasizes that the porous coating must be formed from many layers of small particles to adequately increase surface area and teaches that particles larger than 1,000 angstroms are too large "to be useful in the present invention." (A81 at 27:42-50.)

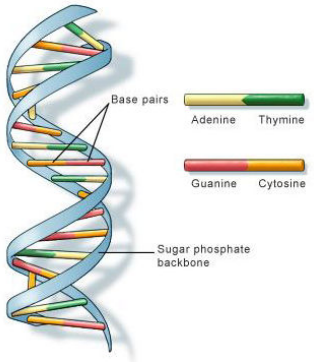
Illumina's accused BeadChip products, by contrast, solve a different problem. It was difficult to attach ligands for millions of genetic mutations to a single prior art array. Illumina solved that problem by creating a two-dimensional array with a single layer of beads with sizes of 12,000 or 20,000 or 31,000 angstroms that sit separately in individual wells on the substrate. Illumina developed expensive scanners to read the optical signal from each bead rather than strengthening it with a porous coating.

Syntrix's patent is either not infringed because it does not cover a single-layer structure, invalid because it describes neither large particles nor a single layer, or both.

STATEMENT OF THE FACTS

I. The Technology: Genetic Testing by Attaching Probe DNA to Surfaces.

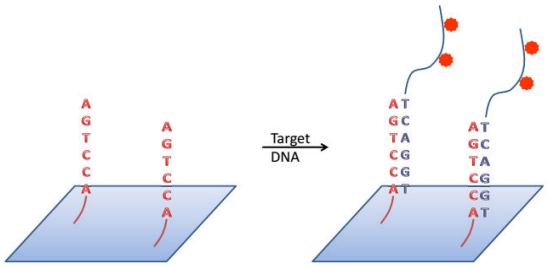
This case is about a tool for genetic testing. Genes are made up of DNA molecules, which are formed from repeating units of four nucleotide bases—adenine (A), thymine (T), cytosine (C), and guanine (G)—that create the familiar double-stranded helix below. (A11278-79.) Each base on one strand forms a bond with a complementary base on the other: A pairs with T, and C pairs with G. (*Id.*)



Many human characteristics—from eye color to predisposition to cancers—depend on the sequence of bases in various parts of our DNA. (A10193-200.) Scientists have identified the sequences responsible for many of these characteristics and developed tests to determine whether someone has a particular sequence. (A10970-71.)

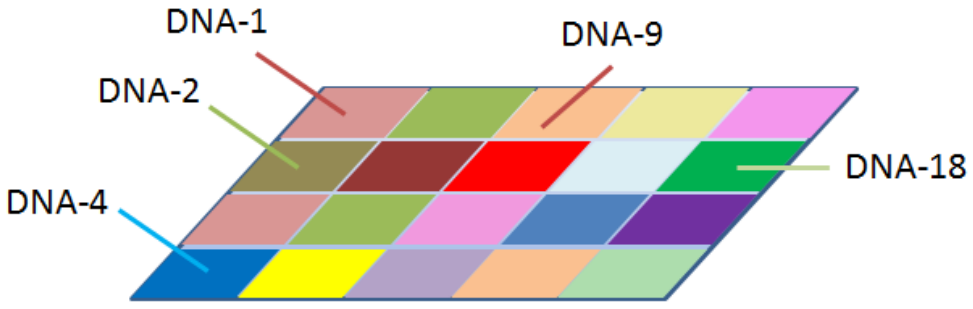
One form of such testing, a "hybridization assay," relies on the base pairing relationships. An example will illustrate. (A11279-80.) Suppose a scientist wants to determine if a patient has a trait associated with a mutation in the six-base sequence "TCAGGT." She can attach a "probe" DNA ligand with the complementary set of base pairs ("AGTCCA") to the surface of a substrate like glass (below, left), and then

expose it under specific reaction conditions to a sample with the patient's DNA. (*Id.*) If the patient's DNA contains the sequence, it will lock onto the probe ligand (below, right). (*Id.*) And if a dye is attached to the patient's DNA, the scientist can look at the substrate (through a scanning microscope) and determine the patient has the sequence if the substrate illuminates where the DNA is attached. (*Id.*)



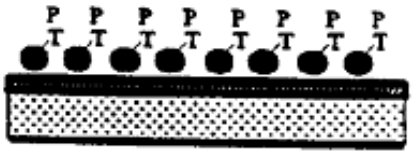
Scientists attach hundreds of thousands of copies of the same probe ligand to strengthen the "signal" emitted from the substrate, making it easier to detect. (*Id.*)

Multiple traits can be tested at once by attaching probe ligands for each trait at different known locations on the substrate, so that fluorescence at a particular X, Y coordinate indicates the patient has the trait associated with that location. (A11281-82.) The example below shows one such configuration, in which an "array" of ligands for different traits are attached at different locations on a checkerboard pattern:



Dense arrays that simultaneously tested for many different traits created at least two problems. (A11281-87.) First, it was hard to selectively attach millions of probe ligands for each gene to a specific tile. (*Id.*) Second, it was hard for the scanners that imaged the arrays to read the results because smaller tiles at each X, Y coordinate also emitted less light—or, in the terminology of the field, emitted weaker signals. (*Id.*)

One approach to address these problems involved attaching the DNA to beads, which made it easier to attach the probe ligands to a desired location. (A10383-85; A1040-124; A3184-93.) For example, a 1997 prior art reference by Seul disclosed attaching DNA to a "planar array" of beads on the substrate:



(A1117.) The beads could have "a wide range of sizes (approximately 100 [a]ngstroms to 10 microns)," *i.e.*, 100-100,000 angstroms. (A1059.) Seul addressed the signal strength issue by holding the beads stationary long enough for a scanner to analyze them in detail:

> Given the fact that the array is readily held stationary by the methods of the present invention, ***image acquisition may be extended to attain robust signal-to-noise for detection of low level signals***. For example, a signal generated by a bead of 10 micron [100,000 angstrom] diameter with at most 10^8 probe-target complexed on the surface of the bead may be detected.

(A1085.)

II. The '682 Patent: Using a Porous Coating With Multiple Layers of Small Particles to Increase Surface Area for Attaching Ligands.

Against this background, the '682 patent-in-suit described a specific problem and proposed a specific solution.

A. The Perceived Problem: A Signal Was Difficult to Detect from Prior Art Arrays Without Using Expensive Equipment.

The '682 patent characterizes existing arrays as inadequate because they generated weak signals that could be detected only by expensive equipment that had to slowly scan the surface to identify which probe ligands lit up. (*See generally* A58-122.) The '682 patent attributes this problem to not being able to pack ligands densely enough on the surface to generate a strong signal:

> The location of bound receptor on the array is determined by detecting photons [*i.e.*, light] or radioactivity. However, ***the surface density of ligand is often low, resulting in the need for costly imaging equipment*** and long image acquisition times.

(A68 at 1:44-48.) One way to increase ligand density was to increase the surface area for attaching ligands, but earlier efforts provided "insufficient" increases:

> Existing techniques for increasing ligand density on a solid support provide insufficient surface area enhancement.

(A68 at 2:1-2.) For example, prior art involving large-pore silicon was inadequate because it provided only a 10-fold increase in surface area over a flat substrate:

> The porous silicon is macroporous with 3 to 5 micron [30,000-50,000 angstrom] diameter pores arranged in parallel and oriented perpendicular to the substrate surface. ***Relative to nanoporous materials, a macroporous configuration has inadequate surface area to significantly increase ligand surface density.*** Although the electrochemically manufactured metal oxide

> membrane has pores as small as 0.2 microns [2,000 angstroms], ***it too provides little surface area enhancement with only a 10-fold increase in surface area*** for each micron [10,000 angstroms] of membrane thickness.

(A68 at 2:7-16.)

The '682 patent therefore concludes that there was a need "for increasing ligand density on a surface" and asserts that its claimed invention addresses that need:

> ***Accordingly, there is a need in the art for methods for increasing ligand density on a surface*** in a manner that is fully compatible with microfabrication. In particular, there is a need for improved articles for use in the detection of macromolecular receptor binding, and the production of ligand arrays by solid-phase synthetic methods. ***The present invention fulfills these needs*** and further provides other related advantages.

(A69 at 3:18-25.)

B. The Proposed Solution: A "Porous Coating" With Small Particles and Multiple Layers to Increase Signal Strength.

The '682 patent proposed solving the signal strength problem by adding a "porous coating" to the substrate and attaching ligands within that coating. The porous coating has a greater surface area within its volume than a flat surface, thereby increasing the number of surface-attached ligands and the resulting signal strength:

> The instant invention overcomes the limitations associated with imaging ligand-receptor binding on low ligand surface densities by providing a support with increased surface area for ligand attachment. Using a porous coating provided herein, imaging may be accomplished rapidly and with less costly equipment.

(A78 at 22:29-34; *see also* A58 at Abstract, A69 at 3:18-25, A70-71 at 6:66-7:7, A102 at 70:21-24, A107 at 79:45-47.) The result, according to the '682 patent, is to eliminate the need for costly scanning equipment. (*Id.*)

The "porous coating" is formed by applying particles in solution to a substrate, and then evaporating the liquid, leaving behind a "gelled network of particles" on the substrate. (A79 at 24:34-41.) A checkerboard pattern with multiple porous coatings on the substrate can be created by placing a mask (36) over the desired region and exposing the assembly to light, permitting removal of the unmasked regions:

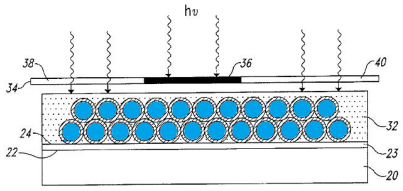


Fig. 1B

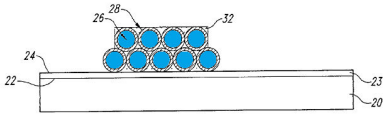


Fig. 1C

As shown in Fig. 1D, the coating particles (26, in blue) may be held together by a polymeric binder (28), and the probes (31, red) may be attached to the porous coating:

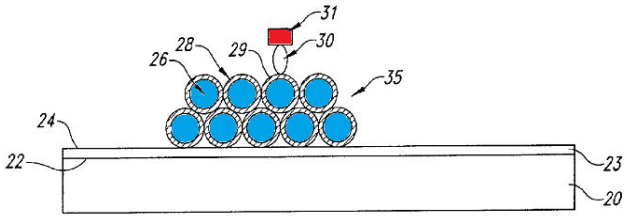


Fig. 1D

(A70 at 5:43-6:3.)

The '682 patent's porous coating must have two key properties to achieve the increased signal strength (and ligand density) of the invention—(1) the particles must be very small, and (2) the "gelled network of particles" must have multiple layers.

First, the specification explains that small particles increase ligand density by increasing available surface area within the coating for ligand attachment:

> A porous coating as described herein provides a high surface area for ligand attachment, thus increasing ligand density. ***The surface area is inversely proportional to average pore size. In turn, the average pore size closely approximates the primary particle size. Thus, the surface area*** and the average pore size of the coating ***may be tailored by the choice of primary particle size*** (*i.e.*, the coating has controlled porosity).

(A81 at 27:35-42.) Although particles "may have any of a variety of sizes and shapes," that "variety" is preferably less than 2,000 angstroms (Å), and, usually, much smaller:

> Particles may have any of a variety of sizes and shapes. Preferably, the particles have a primary particle size of less than 2000 Å, and more preferably less than 1000 Å and still more preferably less than 500 Å. In some embodiments, particles have a primary particle size less than 100 Å, 50 Å, 10 Å or 5 Å.

(A79 at 24:52-57.) The patent's working examples all use porous coatings having 500 angstrom particles. (A104-106 at 74:42-46, 75:37-39, 76:44-77:48, Figs. 2-4; A10391-92.) The inventor used only 200 or 500 angstrom particles in his lab. (A10373.) The patent never mentions any porous coating particle larger than 2,000 angstroms.

In fact, the '682 patent warns against large-particle porous coatings because they lead to reduced surface area and thus reduced ligand density. The specification touts 200 and 500 angstrom particles, while criticizing particles larger than 1,000 angstroms, which yield surface areas "too small to be useful in the present invention":

> For example, a metal oxide with a primary particle size of 500 Å will have a surface area of 50 m^2/g, and a micron [10,000 angstrom] thick coating of such particles will increase the ligand density 100-fold. Similarly, a metal oxide with a primary particle size of 200 Å will have a surface area of 200 m^2/g, and a micron thick coating will increase ligand density 400-fold. In contrast, ***a primary particle size greater than 1000 Å yields porous coatings with surface areas too small to be useful in the present invention.***

(A81 at 27:42-50.) The ligand density increases achieved using small particles are an order of magnitude more than the "only a 10-fold increase in surface area" (and thus at most a 10-fold increase in ligand density) of the prior art. (A68 at 2:11-15.)

Second, the '682 patent ties increased ligand density to a porous coating with multiple layers. The porous coating is comprised of a "gelled network of particles," and the patent defines "gelled network" as "an aggregation of particles linked together to form a porous three-dimensional network." (A73 at 11:20-21.) So the porous coating must be made up of a "three-dimensional network of particles," *i.e.*, multiple layers of particles. The patent also explains that a thicker porous coating—that is, one with more layers of particles—serves the purpose of increasing surface area:

> ***The surface area of a porous coating may be further increased by increasing the thickness of the coating.*** In the instant invention, i[t] has been discovered that a crack-free coating up to 25 μm [250,000 angstroms] thick is possible with a single application of the above dispersion…. In general, however, adequate surface area enhancements are obtained when the thickness is between 2.0 μm and 5.0 μm [20,000-50,000 angstroms].

(A81 at 27:64-28:5.) By contrast, the inventor testified a single layer would increase surface area only 4-fold—less than the increase the patent describes as "insufficient":

> Q. So a single bead compared to flat glass has a 4-fold increase in surface area?
>
> A. Yes.

(A10394; *cf.* A68 at 2:1-16.) The specification uniformly describes porous coatings with multiple layers of particles, most between 20-80 layers. (A10385-92.) No porous coating in the specification has a single layer of particles.

The specification touts the results obtained with porous coatings with multiple layers of small particles. It shows that a set of square-shaped porous coatings of the "present invention" generates a strong signal that appears white (below right), while the prior art signal is so weak that only a few small dots are visible (below left):

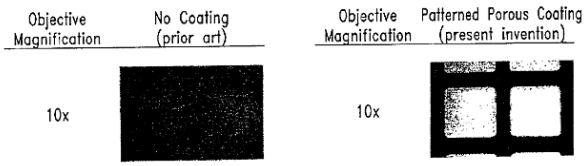


Fig. 4C *Fig. 4D*

(A64 at Fig. 4; A70 at 6:24-30; A10387-89.) It explains that the "present invention" accomplishes this result by increasing ligand density (which results from increased surface area) and thus permits economical scanning with "standard equipment":

> This example demonstrates that ***(1) compared with the image intensity of the prior art, the present invention provides a marked increase in ligand surface density, (2) imaging of ligands on the patterned porous coating is rapid and economical using standard equipment***, (3) the patterned porous coating is compatible with methods of solid-phase synthesis, and (4) the porous coating does not swell or distort during solid-phase synthesis.

(A107 at 79:45-52.) Those porous coatings used 500 angstrom particles stacked at least 40 layers high, (A10387-89), which is consistent with the specification's statement that a porous coating thickness of 20,000-50,000 angstroms is generally required to achieve "adequate surface area enhancements." (A81 at 27:64-28:5.)

The inventor admitted that both multiple layers and small particles are critical: "where you are trying to optimize the amount of signal you are getting, you really want to have many stacks of small particles, because as your particles go down and

your stack goes up, you get increased surface area, much more increased surface area amplification." (A10310.)

C. The Claims: A Porous Coating Comprising a "Three-Dimensional Network of Particles."

Syntrix has asserted claims 1 and 125 against Illumina's BeadChip products. Claim 1 is directed to a substrate with a "porous coating" of "a gelled network of particles," where two or more different compounds (ligands) are attached at "known" locations occupying less than a specified maximum area on the substrate:

> 1. A coated article comprising
>
> a substrate having a continuous ***porous coating*** of substantially uniform thickness, ***wherein the coating comprises a gelled network of particles***,
>
> wherein the porous coating has two or more different compounds attached thereto, and wherein the compounds are attached at known discrete full thickness volumes, each occupying an area on the substrate of less than 1,000,000 μm^2.

(A112 at 89:43-50.)

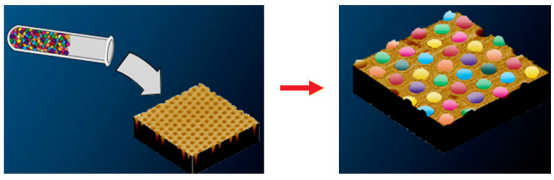
The term "gelled network of particles" requires a "three-dimensional network of particles"—*i.e.*, multiple layers. This is because the specification defines a "gelled network" as "an aggregation of particles linked together to form a porous three-dimensional network." (A73 at 11:20-21.) Incorporating that definition into the claim language, claim 1 thus requires a "three-dimensional network of particles."

Claim 125 covers a method using "a coated article according to claim 1," (A116 at 97:34-42), so it rises and falls with claim 1 for the issues on appeal.

III. Illumina's Technology: Using a Single Layer of Large Beads-in-Wells to Ease Manufacturing, While Developing Better Scanners to Read Them.

Illumina was founded in 1998 based on an invention of Dr. David Walt, a Professor at Tufts University. (A11144-46.) Dr. Walt addressed a different problem than the '682 patent. He was not concerned with increasing signal strength but with easily and inexpensively creating arrays to test for thousands of traits. (A11122-32; A1975 at 3:7-30.) Dr. Walt solved that problem using an array formed with a single layer of large particles, not multiple layers of small particles, as in the '682 patent. (*Id.*)

Dr. Walt's breakthrough was to build an array without selectively attaching ligands at specific locations, a task that had been difficult when even a few traits were involved. (A11122-35; A1975 at 3:17-4:27.) Instead, Dr. Walt attached the probes for each trait to a separate bead bearing a code—such as a unique dye color—that enabled tracking which probes were attached to which bead. (*Id.*) He then randomly placed the beads into wells etched in the surface of a fiberoptic bundle to form an arrangement resembling a Chinese checkerboard. (*Id.*)



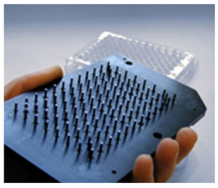
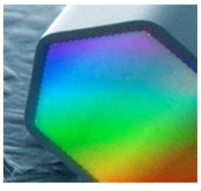
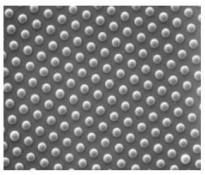
Dr. Walt determined which traits were present in a sample by detecting which beads lit up and then decoding those beads to determine the traits they represent. (*Id.*) In this way, Dr. Walt did not need to control where any particular probe was attached to the array. (*Id.*) The advantage, as Dr. Walt's 1997 patent explains, is that "the burden

is placed on the analysis," *i.e.*, the detecting and decoding, "rather than on sensor manufacture." (A1975 at 4:18-20.)

Dr. Walt took his invention from the university to the marketplace by teaming with John Stuelpnagel and others to co-found Illumina in August 1998. (A11031-32; A10963-64, A10986.) Illumina overcame multiple challenges to create a commercial product. One hurdle was developing a bead decoding process that would allow Illumina to simultaneously identify hundreds of thousands of different probes for different traits. (A3196-3219.) Although Dr. Walt's invention created "a fast and inexpensive process" for attaching compounds, his decoding algorithm could be "difficult and time-consuming to utilize." (A3203 at 4:52-59; 3:28-50.)

Another necessary task was to develop scanners that could detect a signal from each individual bead. After years of work and many millions of dollars, Illumina developed scanners that could read each bead. (A11046; A11206-08.) Each scanner costs around $200,000-$300,000, (A10732; A10969-70), the very type of "costly" equipment the '682 patent sought to avoid. (A68 at 1:46-48; A107 at 79:45-49.)

Illumina launched its first beads-in-wells product, the Sentrix Array Matrix (SAM), in 2003. The images below show the product (left), a close-up of a fiberoptic bundle where the beads are located (middle), and the beads themselves (right):

(A10977-78.) SAM was a major success: it could simultaneously test for around 200,000 traits, had over $100 million in sales, and generated over 70% of the data for the International HapMap Project—a large, worldwide collaboration that generated an extensive map of genetic mutations associated with various diseases. (A11022-28.)

Since Illumina's founding in 1998, it had also explored applying Dr. Walt's beads-in-wells idea to a substrate other than fiberoptic bundles. (A11032-35.) Those efforts culminated in 2005 with the BeadChip products (below left), which incorporate the beads in wells etched in a silicon slide (below middle and right):

Silicon has several advantages: it is less expensive and permits placing the array on a standard-sized surface. (A11063, A11082, A11033-34.) It also accommodates more beads, enabling Illumina to test for more traits simultaneously. (*Id.*) Each BeadChip uses one of three bead sizes—31,000 angstroms, 20,000 angstroms, and 12,000 angstroms. (A10989.) All beads are over ten times larger than the particles the '682 patent said were too large "to be useful," (A81 at 27:42-50), and yield less than the 10-fold surface area increase it deemed "insufficient." (A10394, A68 at 2:1-16.)

The BeadChip has been a major success and tests everything from a patient's risk of cardiovascular disease to a corn crop's resistance to drought. (A10970-77.)

IV. This Lawsuit: Syntrix Stretches Its Patent Beyond What it Describes.

A. Claim Construction and Other Pre-Trial Proceedings.

Syntrix filed this suit in 2010, alleging patent infringement and several state law claims for trade secret misappropriation and breach of contract based on contacts between the companies in 2000. The district court dismissed the state law claims as barred by the statute of limitations, leaving only the patent claim. (A200-08.)

Two claim construction disputes are relevant on appeal. The principal dispute was whether the "gelled network of particles" must have multiple layers. The parties agreed the "gelled network" is "an aggregation of particles linked together to form a porous three-dimensional network." (A73 at 11:20-21.) But they disagreed about the claim scope that results from using that definition. In particular, the court rejected Illumina's proposal, which was that "the requisite 'three-dimensional network' is not satisfied by a planar, two-dimensional configuration of particles.'" (A20-21.)

The parties also disputed whether the claims covered "particles" with an average size greater than 1,000 angstroms. (A22-23.) Illumina argued that they did not, because the patentee's statement that "a primary particle size greater than 1000 Å yields porous coatings with surface areas too small to be useful in the present invention" was a disclaimer. (A81 at 27:48-50.) The court disagreed, (A23), and, although Illumina does not challenge this claim construction on appeal, the consequence of the construction is to render the claims invalid under § 112.

B. The Trial

1. Infringement and Validity.

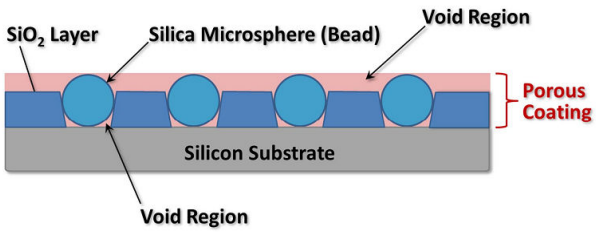
The parties proceeded to trial with the court's constructions. The main issue was whether the single layer of individual beads in discrete wells in the BeadChip products constitutes a "three-dimensional network of particles." On the first morning of trial, the district court expressed second thoughts about its claim construction:

> I want to say that the Court has entered some rulings that I think have been deferred to the trial itself. And among these is the question of whether the term "three-dimensional network" excludes a two-dimension planar array -- or planar array of particles.
>
> While the Court declined to include this limitation during claim [co]nstruction, trial preparation and my increased familiarity with the prior art and technology has made Illumina's position appear to be more meritorious. That is, the term of "three-dimensional" modifies the word "network" and, therefore the gelled network must include multiple layers of particles.

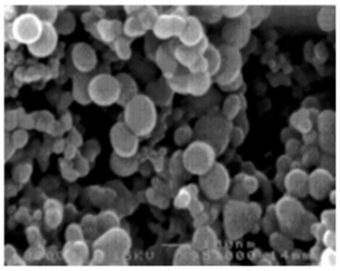
(A10004-05.) The court added that it would listen to the testimony and determine "whether or not that is an issue that can be decided as a matter of law." (A10005-06.)

Syntrix's infringement case conflicted directly with the claim language. Syntrix's inventor testified that the claimed "gelled network of particles" simply requires a "three-dimensional network of ***voids***." (A10292-93, A10359-60.) But then, perhaps concerned with so clearly contravening the claim language, Syntrix's expert tweaked the phraseology and testified that the BeadChip had a "three-dimensional network of particles which have void spaces," (A10699), based on the

combination of the beads (blue spheres), the SiO_2 layer in which the wells are etched (blue trapezoids), and the void space above and below the beads (pink):



(A10620-22, A10698-700.) But neither of these is the "three-dimensional network of particles" that is actually described in the specification and required by the properly construed claim. The '682 patent's porous coating (left) is a glob of many layers of particles, while Illumina's beads-in-wells (right) form a single layer of particles that is at most a planar, two-dimensional network:



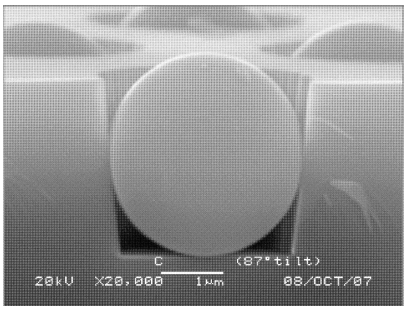


Fig. 3

(A63, A1654; *see also* A11291-96, A11303-26.)

A key dispute on validity was whether Syntrix's assertion of the claims against Illumina's BeadChip renders them invalid for lack of written description because: (1) the patent fails to describe the large 12,000-31,000 angstrom particles that Syntrix now says are covered and, in fact, points away from them; and (2) if the claims cover a

single-layer structure, they are invalid because the patent only describes multi-layer porous coatings and teaches that a single layer of particles would yield an insufficient surface area enhancement. (A11361-63.)

2. Damages.

The parties also presented competing evidence on the appropriate royalty rate. Both agreed the appropriate starting point was the 3% rate in a 1998 license between Tufts University and Illumina for the beads-in-wells patents listing Dr. Walt as an inventor. (A3414-50; A11445-46.) But Syntrix's expert adjusted that rate to 6% and applied it to both the BeadChip and other products sold with it, even though Illumina only pays Tufts royalties on the BeadChip alone. (A10874-78, A10915-17.)

Syntrix's expert testified that he doubled the Tufts 3% rate because Tufts and Dr. Walt had also received stock in Illumina that grew in value to $100 million. (A10846-49.) The undisputed evidence, however, showed that $92 million was Dr. Walt's, and that he obtained the stock through a separate agreement that was unrelated to the Tufts/Illumina patent license. (A10906, A10924, A11151-55; A11462-63.) In particular, Dr. Walt testified without contradiction that he was given the option to purchase the stock because he co-founded Illumina. (*Id.*) He had no rights to the patents when Tufts licensed them to Illumina. (*Id.*) And nothing suggested the stock grant to Dr. Walt was a condition of Tufts licensing the patents. Illumina therefore moved to exclude the expert's testimony regarding the Walt stock under *Daubert*, but the court denied the motion. (A51-52.)

Syntrix's expert offered two other alternative but equally erroneous justifications for doubling the rate from 3% in the Tufts license to 6% in the hypothetical negotiation—(i) a non-peer reviewed study that said that universities generally accept half the royalty rate a commercial entity would demand, and (ii) a comparison between the Walt technology and the patent-in-suit. (A10850-55, A10873-74.) But the study had no tie to the parties, industry, or patent here. And the comparison between the Walt patents and the '682 patent was purely qualitative. After doubling the Tufts rate, the expert made offsetting adjustments for stacking (down 1%) and convoyed sales (up 1%) to reach a 6% rate. (A10874-78.)

C. The Verdict, and the District Court's Post-Trial Rulings.

The jury found claims 1 and 125 of the '682 patent infringed and not invalid, and it awarded $95,795,507 in reasonable royalty damages (applying a 6% rate)—the exact amount advocated by Syntrix. (A178-88.)

Illumina sought JMOL that it does not infringe because the BeadChip's beads-in-wells do not constitute a "three-dimensional network of particles." Illumina reiterated its position that the court should construe the claims to require the "porous coating" to have two or more layers of particles, and, on that basis, find non-infringement as a matter of law. (A15171-85.) The district court denied the motion. Despite having expressed openness to revisiting claim construction when trial began, the court adhered to its prior ruling because it believed Illumina's construction would "add certain limitations to the asserted claims of the patent." (A34.) The court

acknowledged that "expert testimony is unlikely to be reliable in guiding the court's claim construction," but nevertheless concluded that the testimony by Syntrix's expert "was persuasive evidence that the Court's rulings were correct." (*Id.*)

Illumina also sought JMOL that the asserted claims lack adequate written description because the patent fails to describe the large-particle porous coatings that Syntrix has asserted the claims include. (A15191-94.) And Illumina argued that, if the court adhered to its construction of "gelled network of particles," that the claims would lack adequate written description because the specification does not describe a porous coating with a single layer of particles. (*Id.*) The court denied these motions, but it appeared to misunderstand Illumina's argument, and to confuse claim construction and written description:

> In this case, Illumina argues that the patent lacks an adequate ***written description*** because (1) the inventor disclaimed particles less than 1000 Å and (2) the patent does not describe monolayers of beads. Dkt. 363 at 27–30. These ***are both issues of law that were addressed in the Court's claim construction order***. See Dkt. 75 at 11–15. The Court declines to reconsider that order. Therefore, the Court denies Illumina's motion on these issues.

(A38.) In fact, Illumina assumed the correctness of the district court's construction, and, although it mentioned disclaimer, it also explained that, "[i]n any event, the specification must provide support for the full range of the claims." (A15193.)

Finally, the court awarded Syntrix supplemental damages and interest, entered an amended judgment of $115,106,105, (A3-8), and imposed an 8% ongoing royalty through patent expiration in 2019. (A54-57.) This appeal followed.

SUMMARY OF THE ARGUMENT

Lack of Description for a Large-Particle Porous Coating. The claims as construed cover both the small-particle porous coatings the inventor described (*i.e.*, 2,000 angstroms or smaller) and wholly-different large-particle structures (*i.e.*, 12,000-31,000 angstroms). But the patent never describes a large-particle porous coating and, in fact, criticizes particles larger than 1,000 angstroms as producing "surface areas too small to be useful in the present invention." The claims are thus invalid under § 112.

Three-Dimensional Network of Particles. The claim language requires a porous coating made of a "three-dimensional network of particles," not a network of voids. The ordinary meaning of a "three-dimensional network of particles" connotes a structure with particles extending in three dimensions—*i.e.*, multiple layers. The specification confirms this meaning: it relies on multi-layer porous coatings to obtain the required surface area enhancement, repeatedly disclosing structures with 20-80 layers, and describes the maximum surface area enhancement a monolayer could provide as "insufficient." Under the correct construction, Illumina's BeadChip products, which have a single layer of particles, do not infringe as a matter of law. That said, if the claims are construed to cover a single layer of particles, they are invalid because the patent never expressly or inherently describes such a structure.

Damages. If the Court affirms on liability, it should order a new trial on damages because Syntrix erroneously inflated its royalty demand by referencing over $90 million in stock value unrelated to anything Illumina paid for a patent license.

ARGUMENT

I. The Claims Are Invalid Because the Specification Does Not Describe a Large-Particle Coating Like Those Syntrix Accuses of Infringement.

A. Precedent Prohibits a Patentee from Claiming a Class of Structures that Are Not Described in the Specification.

The written description requirement ensures that an inventor's patent rights do not "overreach the scope of [his] contribution to the field of art as described in the patent specification." *Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1353 (Fed. Cir. 2011). "[T]he applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and demonstrate that by disclosure in the specification of the patent." *Id.* at 1347. The Court determines compliance by "an objective inquiry into the four corners of the specification" and can find the patent invalid "based solely on the face of the patent specification." *Id.* Written description is a fact issue reviewed for substantial evidence. *Id.*

This case resembles a string of others in which this Court has held that the claims lacked adequate written description as a matter of law. In each case, the patent described only a single class of items, but was then asserted to cover a different class of items that were nowhere described in the patent.

For example, in *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473 (Fed. Cir. 1998), a patent for a sectional sofa with reclining seats showed the recliner controls only in a center console of the sofa, but the patentee read the claim to cover any

location for the controls, including controls outside a console. *Id.* at 1478-80. The court held the claims invalid as a matter of law because the specification "provides for only the most minor variation in the location of the controls" within the console and "[n]o similar variation beyond the console is even suggested." *Id.* at 1479. Moreover, the specification showed that "locating the controls anywhere but on the console is outside the stated purpose of the invention" by stating that an "object of the present invention is to provide . . . a console positioned between [the reclining seats] that accommodates the controls for both of the reclining seats." *Id.*

Likewise, *Tronzo v. Biomet, Inc.*, 156 F.3d 1154 (Fed. Cir. 1998), dealt with a patent where the specification described a prosthetic hip socket with a cup having conical shape but the claims covered sockets with a cup of any shape. The Court found the claims invalid as a matter of law because the specification never mentioned anything other than a conical cup and "specifically distinguishes the prior art as inferior and touts the advantages of the conical shape of the '589 cup." *Id.* at 1159. "Such statements make clear that the '589 patent discloses only conical shaped cups and nothing broader." *Id.*

Another example is *Lizardtech, Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336 (Fed. Cir. 2005), which determined that claims covering all methods for creating a "seamless digital wavelet transform" for image compression were invalid as a matter of law where the specification disclosed only one, specific method for doing so. *Id.* at 1344. The problem, once again, was that the specification was "directed at describing

a particular method for creating a seamless DWT, as opposed to using the disfavored, nonseamless prior art, and it teaches only that method of creating a seamless array." *Id.* at 1345. The description did not support claims covering different ways of creating a seamless DWT because "to hold otherwise would violate the Supreme Court's directive that '[i]t seems to us that nothing can be more just and fair, both to the patentee and the public, than that the former should understand, and correctly describe, just what he has invented, and for what he claims a patent.'" *Id.* at 1346.

Still another example is *ICU Medical, Inc. v. Alaris Medical Systems, Inc.*, 558 F.3d 1368 (Fed. Cir. 2009). There, the patent described a medical valve in which fluid flowed through a sharp, pointed spike, while the claims were directed to a valve that could use any structure for transmitting fluid, including a "spikeless" valve, such as the accused product, which had a short, blunt tube. *Id.* at 1377-79. The Court held the claims invalid as a matter of law because they covered "valves that operate with a spike and those that operate without a spike" but "the specification describes only medical valves with spikes." *Id.* at 1378.

A final example is *Anascape, Ltd. v. Nintendo of Am. Inc.*, 601 F.3d 1333 (Fed. Cir. 2010). The specification consistently described videogame controllers with only a single input that operated in six degrees of freedom, yet the claims also covered controllers with multiple inputs that operated in six degrees of freedom. *Id.* at 1335-41. That specification suffered from similar problems as those in prior cases—the Background, Abstract, and Summary sections all referred to a single input controller;

the specification said that a "primary object of the invention" was to provide a controller with a single input; the drawings all showed a single input controller; and there were "over twenty explicit statements that the invention is directed to a single input member that is operable in six degrees of freedom." *Id.* at 1335-36. The specification also attributed "significant disadvantages" to prior art controllers with multiple inputs. *Id.* at 1337. The Court thus concluded that "the only reasonable reading" was that the specification "describes only a controller having a single input member operable in six degrees of freedom." *Id.* at 1340.

B. The '682 Patent Does Not Describe a Large-Particle Coating Like Those Covered by the Claims As Construed.

The claims here suffer from the same deficiencies as those just discussed. The '682 patent describes a specific class of small-particle porous coatings (with particles that are at most 2,000 angstroms). But Syntrix now asserts the claims against a wholly different set of large-particle structures (like the 12,000-31,000 angstroms beads-in-wells of Illumina's BeadChip products). The specification does not describe porous coatings formed of these types of large particles, and, in fact, teaches that particles of that size result in surface area increases that are too small "to be useful in the present invention." (A81 at 27:42-50.) The claims are thus invalid as a matter of law.

First, the specification never describes a large-particle coating. It never mentions a porous coating particle bigger than 2,000 angstroms, and it explains that particle size should be "preferably" less than 2,000 angstroms, "more preferably" less

than 1,000 angstroms, and "still more preferably less than 500 angstroms." (A79 at 24:52-57.) No variation above 2,000 angstroms is ever suggested, and the specification certainly never suggests a particle size of 31,000 angstroms—over an order of magnitude larger. Large-particle porous coatings are a wholly different set of structures than the small-particle porous coatings described in the specification, just like the "spikeless" medical valves in *ICU Medical*, the multiple-input controllers in *Anascape*, the sofas with controls outside the console in *Gentry Gallery*, and the other ways of creating a digital wavelet transform in *LizardTech*.

Second, and relatedly, the specification makes clear that large-particle porous coatings are outside the scope of the invention. The invention's purpose is to increase signal strength by using a "porous coating" formed of many layers of small particles to increase ligand density by increasing surface area. (A68-69 at 1:44-48, 3:18-25, 2:1-16; A81 at 27:35-42.) The specification touts the increased surface area obtained using 500 and 200 angstrom particles, which produced 100- and 400-fold ligand density (and thus surface area) increases per micron (10,000 angstroms) of coating thickness, unlike the "insufficient" 10-fold increase of the prior art. (A68-107 at 27:42-50, 2:1-16, 6:24-30, 78:25-79:52, Figs. 4A-4F; A10387-89.) And the specification expressly discourages using particles larger than 1,000 angstroms: "a primary particle size greater than 1000 Å yields porous coatings with surface areas too small to be useful in the present invention." (A81 at 27:42-50.) That statement makes perfect sense given the distinction the '682 patent draws with the prior art. For

example, a single layer of large 10,000 angstrom (1 micron) particles that are touching one another would yield at most a 4-fold increase in surface area per micron of coating thickness. (A10394.) That is less than half of the already "insufficient" 10-fold increase of the prior art. (A68 at 2:1-16.) The large beads-in-wells of Illumina's products (which are 12,000-31,000 angstroms and are spaced apart) yield even smaller surface area increases.

These disclosures demonstrate that the '682 invention is limited to the small-particle coatings actually described. Here, as in *Gentry Gallery*, *Tronzo*, *LizardTech*, and *Anascape*, the invention's purpose and purported distinction over the prior art also demonstrated its limited scope. Moreover, the specification here shows that small particle size is tied to the invention as a whole—just as in *Anascape*, the '682 patent's emphasis on small particles and increasing surface area appears at least a dozen times, including in the Abstract and the Background of the Invention. (A58-122 at Abstract, 1:44-48, 2:1-15, 3:18-25, 6:65-7:7, 22:29-32, 27:35-50, 70:21-24, 78:24-30, 79:31-34, 79:45-52, 81:13-22.) The '682 patent always associates the increased surface area achieved using small particles with "the present invention," "the instant invention," or "the coatings described herein." (*Id.*) So increasing surface area with a small-particle coating is not just one advantage of a broader invention—it ***is*** the invention. The asserted claims, which also cover a wholly different class of large-particle structures, are thus invalid as a matter of law.

C. Syntrix Failed to Identify Any Description of Large-Particle Coatings to Support its Claims.

Nothing relied upon by Syntrix or the district court shows a description of large coating particles, like the 12,000-31,000 angstrom beads in the accused products.

The district court's sole basis for denying JMOL was its erroneous belief that written description had been resolved at claim construction because the patentee had not "disclaimed" particles larger than 1,000 angstroms. (A38.) But claim construction and written description are two different issues. Even if the patentee did not disclaim particles larger than 1,000 angstroms, that does not mean he adequately described it. *See, e.g.*, *Anascape*, 601 F.3d at 1339 ("A patentee is not deemed to disclaim every variant that it does not mention. However, neither is a patentee presumed to support variants that are not described."). A claim can still be invalid under § 112 even after the court has broadly construed it. *See, e.g.*, *LizardTech*, 424 F.3d at 1344-47 (refusing to construe a claim to be limited to a single way of generating a seamless DWT, but then finding it lacked written description); *Atlantic Research Marketing Sys., Inc. v. Troy*, 659 F.3d 1345, 1354-56 (Fed. Cir. 2011) (construing claims to cover a handguard accessory for a rifle that is completely supported by the barrel nut but then invalidating them because this configuration was not described); *Liebel-Flarsheim Co. v. Medrad, Inc.*, 481 F.3d 1371, 1380 (Fed. Cir. 2007) (invalidating claims for lack of enablement after having previously refused to limit them to cover only injectors with a "jacketless system").

Syntrix identified two parts of the specification that it says describe a large-particle porous coating, but neither does. First, Syntrix's inventor pointed to a sentence that says the porous coating's particles "may have any of a variety of sizes and shapes." (A79 at 24:52-53; A10356-57, A10301-03.) But this passage is referring to the small-particle porous coatings of 2,000 angstroms or less. It does not say the particles can be "any size"—it says they can be "any of a variety of sizes." The next few sentences specify what that "variety of sizes" is, with 2,000 angstroms preferably being the largest, and the stronger preference being for something much smaller (even orders of magnitude smaller):

> Particles may have any of a variety of sizes and shapes. Preferably, the particles have a primary particle size of less than 2000 Å, and more preferably less than 1000 Å and still more preferably less than 500 Å. In some embodiments, particles have a primary particle size less than 100 Å, 50 Å, 10 Å or 5 Å.

(A79 at 24:52-57.) When read in context, this passage neither expressly nor inherently describes a particle larger than 2,000 angstroms, much less a particle size over 15 times larger, as in Illumina's accused 31,000 angstrom BeadChip.

Indeed, if the '682 patent intended to describe large particles, it would have said so. Other prior art patents that sought to cover large beads explicitly identified those sizes. For example, the Seul application explicitly described particles as large as 10 microns (100,000 angstroms), (A1059), and the Walt patent gave a range of particles as large as 1 mm (10 million angstroms). (A1976 at 5:29-32.) Instead, the '682 specification picked small particles because the large particles of the prior art were

inconsistent with the '682 patent's stated purpose of increasing signal strength to eliminate costly scanning equipment and reduce acquisition times. (A68-69 at 1:44-48, 3:18-25; A107 at 79:45-52.) By contrast, prior art like Seul was content to deal with a weaker signal associated with larger particles by holding them stationary and relying on a longer signal acquisition time. (A1085.) And, when commercializing the Walt technology, Illumina developed better scanners that can resolve the signal from each individual bead. (A11046; A11206-08; A10732; A10969-70.)

Second, Syntrix's inventor and expert both pointed to a passage that is irrelevant because it does not discuss the particles of the claimed porous coating. The passage defines what it means for a "compound" to be "'attached' to a substrate surface," explains that one can use "magnetism" to attach compounds to a "***substrate***," adds that "micron-scale and smaller ***magnetic affinity particles*** may be used," and cites articles referring to particles between 1-100 microns (*i.e.*, 10,000-1,000,000 angstroms) (emphasis added). (A71 at 8:44-67; A11622-23, A11718-22.) None of this refers to the size of the claimed ***porous coating particles***. In fact, Syntrix itself argued during claim construction that this is "a portion of the specification that defines attachment of compounds to a substrate surface—not a porous coating as the claim requires." (A15113; *see also* A15140.) The court accepted Syntrix's argument and adopted its construction of "attached to" based on this distinction. (A24.) Having successfully argued at claim construction that the passage isn't relevant to porous coatings, Syntrix cannot now argue that it is.

There was also debate below about whether the claims themselves impose an upper limit on the coating's particle size. (A11624-25.) But everyone agrees that the claims, as construed, cover large-particle structures, including 31,000 angstrom beads. They are invalid because the patent never describes this type of large-particle coating.

The bottom line is that none of Syntrix's arguments show the required express or inherent description of a large-particle porous coating. Testimony given 15 years after the filing date cannot supply what is missing from the four corners of the specification. *See, e.g., Tronzo*, 156 F.3d at 1159 (discounting expert testimony that a section of the patent provided written description support where that section "served the narrow purpose of reviewing the prior art and did not describe the invention"); *Anascape*, 601 F.3d at 1339 (discounting expert testimony and explaining that its "conclusion is not supported by any evidence at all, and cannot override the objective content of these documents"). And Syntrix's passages must also be read in the context of the rest of the specification, which consistently demonstrates that large particles like Illumina's 12,000-31,000 angstrom beads are outside the described invention.

II. The Judgment Should Be Reversed or Vacated Based on the Proper Treatment of the "Three-Dimensional Network of Particles" Limitation.

A. The Court Should Correct the Claim Construction and Either Enter Judgment of Non-Infringement, or, at a Minimum, Vacate.

The claims require that the porous coating be made of a "gelled network of particles." The specification defines the term "gelled network" as "an aggregation of

particles linked together to form a porous three-dimensional network." (A73 at 11:20-21.) Incorporating that definition into the claim language demonstrates that the claimed "gelled network of particles" must be a "three-dimensional network of particles." A three-dimensional network of particles has multiple layers. The district court therefore erred in construing the claims to permit Syntrix to argue they covered a single layer of particles (or, as the inventor described it at trial, a "network of voids"). This Court should correct the district court's construction and enter judgment of non-infringement.

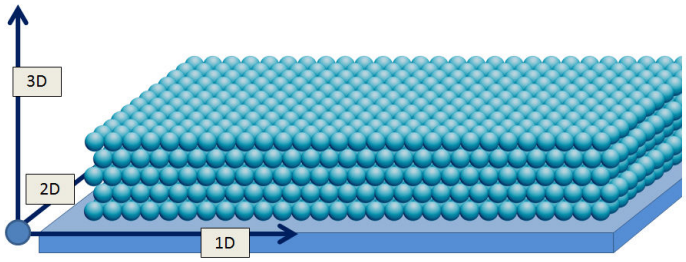
1. The Claims Require a "Three-Dimensional Network of Particles," *i.e.*, a Network With Multiple Layers of Particles.

Claim construction is an issue of law that this Court reviews *de novo*. *Lighting Ballast Control LLC v. Philips Elecs. N. Am. Corp.*, 2014 WL 667499 (Fed. Cir. Feb. 21, 2014) (*en banc*). Claim construction turns primarily on the intrinsic evidence—especially the claims themselves and the specification. *Philips v. AWH Corp.*, 415 F.3d 1303, 1312-17 (Fed. Cir. 2005) (*en banc*). "[E]xtrinsic evidence may be useful to the court, but it is unlikely to result in a reliable interpretation of patent claim scope unless considered in the context of the intrinsic evidence." *Id.* at 1319. The intrinsic evidence here demonstrates that the claimed "three-dimensional network of particles" must have multiple layers.

As an initial matter, the claim requires a "three-dimensional network of particles," not merely a "network of voids." The claims recite a porous coating

comprised of "a gelled network of particles." (A112 at 89:43-50; A116 at 97:34-42.) It is undisputed that the specification defines the term "gelled network." (A73 at 11:20-21.) So, by substituting that undisputed glossary definition into the rest of the claim language, the claimed "gelled network of particles" must be "an aggregation of particles linked together to form a porous three-dimensional network of particles." The claim thus requires "a gelled network of particles" that is three-dimensional. It is not enough that the particles themselves are three-dimensional. Nor is it enough that the void spaces around the particles are three-dimensional.

With that initial understanding, the remaining analysis is straightforward. The plain meaning of a "three-dimensional network of particles," illustrated by the demonstrative below, is a network of particles with multiple layers.



A "one-dimensional network of particles" is an aggregation of linked particles that extends in a single direction. A "two-dimensional network of particles" is an aggregation of linked particles that extends in two directions—like the single, "planar" layer of particles in the prior art Seul reference. (A1117.) A "three-dimensional network of particles" is an aggregation of linked particles that extends in all three dimensions and must therefore have multiple layers.

The specification, which is "the single best guide to the meaning of a disputed term," confirms this understanding. *Phillips*, 415 F.3d at 1315. The '682 patent uniformly depicts the "three-dimensional network of particles" as having multiple layers. Figure 1 shows a drawing of the porous coating with 2 layers, Figures 2 and 3 show a porous coating with at least 40 layers, and all the working examples are porous coatings with between 20-80 layers. (A58-122 at 5:42-6:23, 74:37-45, 76:44-45, 77:40-51, 79:25-44; Figs. 1, 2, 3, 4B, 4D, 4F; A10385-92.) Indeed, Syntrix's expert admitted the specification never shows an example of a three-dimensional network of particles with a single layer. (A10699.) The specification's consistent depiction of the three-dimensional network of particles as including multiple layers strongly suggests that the term refers to just that—a network with multiple layers of particles. *See, e.g.*, *Retractable Tech. v. Becton, Dickson, & Co.*, 653 F.3d 1296, 1304-05 (Fed. Cir. 2011) (construing the term "body" to require a one-piece body where "each figure that depicts a syringe body shows a one-piece body"); *Hologic, Inc. v. SenoRx, Inc.*, 639 F.3d 1329, 1338 (Fed. Cir. 2011) (construing a claim term to cover a particular arrangement where "the specification, including the figures, consistently and exclusively" disclosed that arrangement, which was "clearly what the inventors of the . . . patent conceived of").

What is more, the invention's stated purpose—significantly increasing surface area to significantly increase signal strength—requires multiple layers. The inventor admitted that using a single layer of particles would, at most, increase surface area 4-fold. (A10394.) But the patent describes the 10-fold increase accomplished by the

prior art as "insufficient." (A68 at 2:1-15.) So, if the claimed "three-dimensional network of particles" covered a single layer of particles, it would cover what the specification deemed insufficient, which strongly counsels against such a construction. *See, e.g.*, *LizardTech*, 424 F.3d at 1343-44 ("[I]t would be peculiar for the claims to cover prior art that suffers from precisely the same problems that the specification focuses on solving.").

Likewise, the specification's statement that one can increase the porous coating's surface area by decreasing particle size necessarily assumes that the coating has multiple layers. (A81 at 27:35-50.) Particle size has no impact on the relative surface area increase from adding a porous coating if the porous coating is made from a single layer of particles. (A11314-18; A10362-63.) Although the explanation requires some math, neither the math nor the conclusion was disputed below. The surface area increase that a porous coating generates over a flat substrate is the coating's surface area divided by the surface area of the substrate that the coating covers. (A11315-16.) Both of these quantities are proportional to the radius squared (r^2) of the porous coating particles. (*Id.*) When you divide one by the other, the result is a constant that does not depend on particle size. (*Id.*) Therefore, the specification's statement that one can obtain better surface area enhancements from the "porous coating as described herein" by using smaller particles demonstrates that the porous coating must be made of multiple layers of particles. (A81 at 27:35-42.)

Indeed, the specification makes clear that the more layers of particles, the better. Thicker coatings provide greater increases in surface area. (A81 at 27:64-28:5.) The specification touts the performance of the "present invention" compared to the prior art by showing images obtained with a porous coating with at least 40 layers of particles, (A106-107 at 78:25-79:52; A10387-89), and boasts that the claimed porous coating can be made much thicker (up to 25 μm) without cracking than porous coatings in the prior art. (A70-71 at 6:66-7:7, A81 at 27:64-28:1, A94 at 53:14-19; A68 at 2:46-51.) Nothing suggests a single layer is within the scope of the invention, and, as discussed in the next section, the claims would lack written description if construed to cover a single layer. That confirms the correctness of Illumina's construction. *Bayer CropScience AG v. Dow AgroSciences LLC*, 728 F.3d 1324, 1331 (Fed. Cir. 2013) ("[T]he significant invalidity troubles that accompany Bayer's construction substantiate our rejection of it.").

The district court rejected Illumina's construction because it thought the specification does not disclaim a porous coating with a single layer of particles and was concerned about reading a limitation into the claims. (A20-22, A34.) But "[i]n reviewing the intrinsic record to construe the claims, we strive to capture the scope of the actual invention" and do not "allow the claim language to become divorced from what the specification conveys is the invention." *Retractable Techs.*, 653 F.3d at 1305. Disclaimer or not, the plain meaning of a "three-dimensional network of particles"

(both on its own and in light of the specification) requires multiple layers of particles. The danger here is reading a limitation out of the claims, not into them.

Syntrix's justifications for stretching the claims beyond porous coatings with multiple layers are similarly insufficient. Syntrix tried to create a disclosure of a single layer by combining the following two parts of the specification, which give potential ranges for the porous coating's particle size and thickness:

- "The thickness of the porous coating may vary over a wide range (0.05 to 25 microns [500 Å -250,000 Å, *i.e.*, angstroms])." (A81 at 27:30-32.)

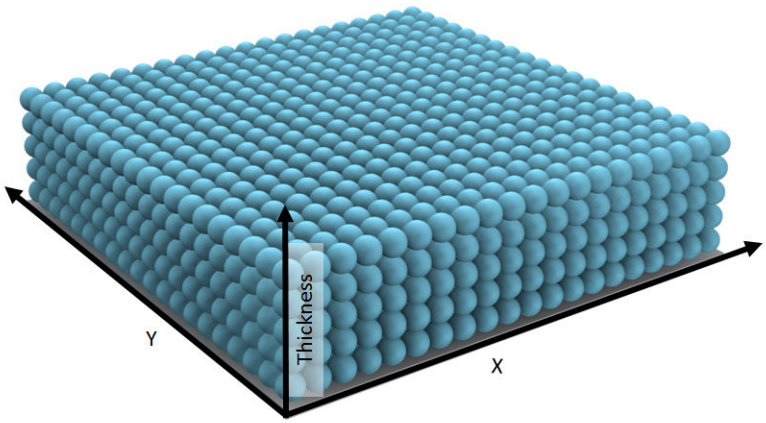
- "Particles may have any of a variety of sizes and shapes. Preferably, the particles have a primary particle size of less than 2000 Å, and more preferably less than 1000 Å and still more preferably less than 500 Å. In some embodiments, particles have a primary particle size less than 100 Å, 50 Å, 10 Å or 5 Å." (A79 at 24:52-57.)

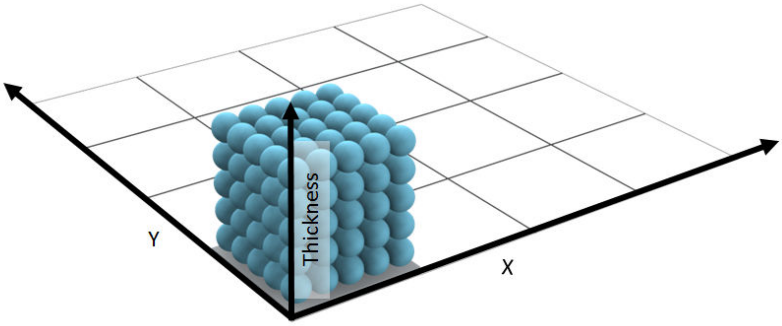
Syntrix argued that combining the thinnest porous coating (500 angstroms) with a particle size on the mid-to-high end of the range (500 angstroms) would yield a single-layer porous coating. (A10538-39, A10727-28, A11625-28.) But the specification itself never articulates such a combination.

In fact, these ranges point away from a single layer of particles. The smallest porous coating thickness (500 angstroms) is two orders of magnitude larger than the thickness of a single layer of the smallest particle (5 angstrom). The smallest thickness is also more than the thickness of a single layer of most of the preferred particle sizes (i.e., 5, 10, 50, 100, and even 200 angstroms). So porous coatings made from particles of these sizes must have multiple layers of particles. And the disconnect between the

smallest disclosed thickness and smallest disclosed particle size suggests that the specification only contemplated the porous coating having multiple layers of particles.

Syntrix also incorrectly relied on a part of the specification discussing how much ***area*** the porous coating covers on the substrate to argue that the '682 patent discloses a single-layer porous coating. (A11628-29, A11654-56; A81 at 28:27-35.) But the number of layers in the porous coating depends on its ***thickness***, not the ***area*** it covers. The demonstratives below illustrate that point—both show porous coatings with the same thickness (and thus the same number of layers), but the area of the second porous coating is smaller:





The part of the specification relied upon by Syntrix does not relate to the coating's thickness. Instead, it explains that multiple, discrete coatings can be applied to the same substrate and that the area each coating covers on the substrate is a factor of the size of the individual particles:

> The resulting article preferably comprises greater than 10^3, 10^4, 10^5, or 10^6 porous coatings, and each coating preferably has an area between about 1 cm^2 and 10^{-12} cm^2. ***In some embodiments, the area occupied by each porous coating may be extremely small, being limited by the size of the individual metal oxide particles.*** For example, a porous coating may occupy an area less than about 10^{-1} cm^2, 10^{-2} cm^2, 10^{-3} cm^2, 10^{-4} cm^2, 10^{-5} cm^2, 10^{-6} cm^2, or 10^8 cm^2, or 10^{-12} cm^2.

(A81 at 28:27-35.) This discussion has nothing to do with the porous coating's thickness or how many layers of particles it includes. Instead, the passage is discussing the limits on the X-Y area of each checkerboard tile of the porous coating.

Finally, Syntrix's extrinsic inventor and expert testimony about what constitutes a "three-dimensional network" deserves no weight because it contradicts the claim language and the specification. *Phillips*, 415 F.3d at 1319. It is certainly no more persuasive than Illumina's expert testimony, which was grounded in the claims and the specification. (A11291-96, A11303-26.) The Court should thus adopt Illumina's proposed construction.

2. The Court Should Enter Judgment of Non-Infringement Under the Correct Construction.

With the correct construction in place, the Court must next determine its effect on the judgment. The parties have already had a trial, and Illumina renewed its claim

construction arguments post-trial, asking the district court to fix its construction and grant judgment as a matter of law of non-infringement. (A15171-85.) It is undisputed that Illumina's BeadChip products do not have multiple layers of particles—they have, at most, a single layer of beads. (A10620; A10573-74; A1654-55.) Indeed, the stark difference between the multi-layer porous coating of the '682 patent (left) and the single layer of beads in individual wells of Illumina's BeadChip (right) is apparent from the images below.

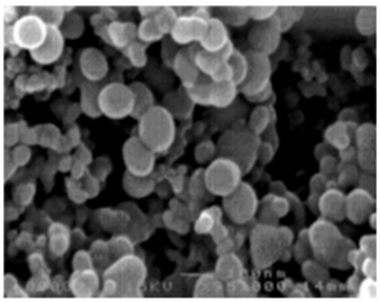
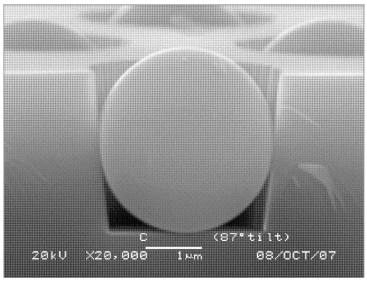


Fig. 3



(A63, A1654; *see also* A11291-96, A11303-26.)

Therefore, if this Court determines that the claims require a porous coating with multiple layers of particles, there can be no infringement, and this Court should reverse and remand with directions to enter judgment of non-infringement. *See, e.g., Cordis Corp. v. Boston Scientific Corp.*, 658 F. 3d 1347, 1357-58 (Fed. Cir. 2011) (affirming entry of judgment of non-infringement after the district court clarified its claim construction post-trial). At a minimum, any change to the construction would require the Court to vacate and remand to determine the impact on infringement.

B. The Claims Are Invalid Under the District Court's Construction Because a Single-Layer Porous Coating Is Never Described.

If this Court were to affirm the district court's construction of "gelled network" of particles, then it should still reverse the judgment because the claims would be invalid as a matter of law for lack of written description. There is no description of a porous coating with a single layer of particles in the specification.

The written description problem here is similar to the problem discussed above with particle size. The purpose of the invention is to increase signal strength by increasing the available surface area to attach ligands. (A78 at 22:29-34; *see also* A58 at Abstract, A69 at 3:18-25, A70-71 at 6:66-7:7, A102 at 70:21-24, A107 at 79:45-47.) The patent describes accomplishing that goal using a porous coating with many layers of small particles. (*Id.*) Every described embodiment includes two or more layers of particles. Most of the specific examples use at least 20-80 layers, (A10391), and Figure 4 compares an at least 40-layer coating of the "present invention" to the "prior art" to show how the invention increases signal strength. (A10387-89.)

Beyond not disclosing a porous coating with a single layer, the specification warns the skilled artisan against it. Using a single layer of particles would, at best, result in only a 4-fold increase in surface area, much less than the 10-fold increase the specification labels "insufficient." (A10394, A68 at 2:1-15.) By contrast, the specification explicitly encourages using more layers because that further increases available surface area. (A81 at 27:42-48, 27:64-28:5.) The message these disclosures

would convey to the skilled artisan is that a porous coating with a single layer of particles is "insufficient" and outside the scope of the invention because it suffers from the same problem the '682 patent thought plagued the prior art.

So claims covering a single layer of particles are invalid for the same reasons discussed in *Anascape*, *Tronzo*, *LizardTech*, *Gentry Gallery*, and the other cases cited above: (1) the specification never actually describes a porous coating with a single layer of particles, and (2) the purpose of the invention and its distinction over the prior art explicitly discourage using a single layer of particles.

There is no legally relevant evidence that is sufficient to uphold the claims' validity. The district court erred in denying JMOL by confusing claim construction with written description, just as it did when analyzing the written description issue regarding particle size. (A38.) Moreover, the court's claim construction order never identified a description of a single layer of particles anywhere in the '682 patent. (A20-21; *see also* A34-36.)

For its part, Syntrix presented no evidence that the '682 patent expressly or inherently discloses a porous coating made of a single layer of particles. Instead, Syntrix attempted to create a disclosure by combining various parts of the '682 patent—*e.g.*, the ranges of coating thickness and particle size, (A11625-28), and the passage about the coating's surface area. (A11628-29, A11654-56.) Those attempts were legally insufficient under this Court's precedent. *See, e.g.*, *ICU Medical*, 558 F.3d at 1376-79; *Tronzo*, 156 F.3d at 1159. Neither passage is an express description of a

single-layer porous coating. And neither passage inherently describes a single layer porous coating because the skilled artisan would not "necessarily" combine or interpret them as Syntrix urges. Indeed, the passage regarding the coating's surface area does not relate to its thickness at all, rendering it irrelevant as a matter of law. *See, e.g., Tronzo*, 156 F.3d at 1159; *Anascape*, 601 F.3d at 1339. The claims, as construed by the district court, are thus invalid under § 112.

III. If Liability is Affirmed, the Court Should Vacate the Damages Award.

This Court should, at a minimum, vacate the damages award because it was infected by inadmissible expert testimony regarding Dr. Walt's receipt of stock that was unrelated to any patent rights that Tufts licensed to Illumina. "The patentee bears the burden of proving damages." *Uniloc USA, Inc. v. Microsoft Corp.*, 632 F.3d 1292, 1315 (Fed. Cir. 2011). This Court reviews the admission of expert testimony on damages for abuse of discretion. *Id.* at 1315-18. Evidence that is legally irrelevant is not properly admissible. *Id.*; *see also* FED. R. EVID. 402, 702.

The Walt stock is relevant only if it were part of the compensation that Illumina paid to induce Tufts to license the patents. Both parties started their royalty analysis with the Tufts-Illumina license, which was relevant under *Georgia-Pacific* Factor 2: "the rates paid by the licensee for the use of other patents comparable to the patent in suit." *Lucent Techs., Inc. v. Gateway, Inc.*, 580 F. 3d 1301, 1325 (Fed. Cir. 2009). Syntrix doubled the 3% rate in the Tufts license when determining a royalty for the patent-in-suit because Tufts and Dr. Walt supposedly received $100 million in stock,

in addition to the 3% royalty. (A10846-49.) All but $7.7 million of the stock went to Dr. Walt, not Tufts, (A11463), yet Syntrix's expert speculated that Dr. Walt's separate stock purchase was "part of the overall transaction between Tufts and Illumina," and included the Walt stock on that basis. (A10847.)

But there was no evidence that Dr. Walt received the stock as compensation for patent rights—the only pertinent issue under *Georgia-Pacific* Factor 2. Indeed, Dr. Walt no longer had any rights to the patents: he had already assigned them to Tufts before being asked to purchase the stock as a member of Illumina's founding team. (A3414.) There was no evidence that Dr. Walt could (or did) influence Tufts's licensing behavior, much less that either he or Tufts insisted that Illumina permit him to purchase stock as a condition of the license. Indeed, Dr. Walt testified, without contradiction, that he purchased the stock separate from his relationship with Tufts:

> Q. Now, as a founder of the company, is it true you receive[d] stock?
>
> A. Yes, that's correct.
>
> Q. Explain that, how did that come about?
>
> A. So venture capital companies, when they make investments, they oftentimes want the inventor of the technology to be associated with the company in a way that's a meaningful participation. They want the -- as much attention from that individual as possible. And so the inventor's interests are ideally aligned with the interests of the company. And so what typically will happen is that ***outside of the relationship that I have with my university, they ask me to be part of the founding team of the company, the scientific founder of the company; and in exchange for that, they gave me a grant of stock*** that, at the time, was just a small company, just a piece of paper.

Q. Was it a grant or did you have to write a check?

A. I had to write a check.

Q. How many shares and what price?

A. There were a million shares at a penny a share.

(A11154-55.)

Despite this evidentiary void, Syntrix relied on the Walt stock to inflate the royalty in a similar way to the methodologies rejected in *ResQNet.com v. Lansa, Inc.*, 594 F.3d 860 (Fed. Cir. 2009) and *Lucent*, 580 F.3d at 1327-32. In *ResQNet*, the Court vacated a damages award because the patentee relied on rates from bundled licenses that related, not to patent rights, but to "finished software products and source code, as well as services such as training, maintenance, marketing, and upgrades." *ResQNet*, 594 F.3d at 870. Because the patentee "did not provide any link" between licenses involving those non-patented services and the patent-in-suit, the Court determined it was impossible to "understand how the [fact finder] could have adequately evaluated the probative value of [the] agreements." *Id.* at 871. Likewise, in *Lucent,* this Court vacated a damages award where the patentee relied on some licenses that were "radically different from the hypothetical agreement under consideration" and others where "we are simply unable to ascertain from the evidence presented the subject matter of the agreements." *Lucent*, 580 F.3d at 1327-28.

Syntrix's use of the Walt stock reflects a similar error to those cases. Syntrix presented no evidence to link Dr. Walt's stock grant to a payment for patent rights.

And the only evidence regarding the stock grant was Dr. Walt's testimony that it was for his role as a founder, not for patent rights—just like the re-bundling licenses in *ResQNet*, which reflected payments for unpatented services, and the licenses in *Lucent*, where there was no evidence tying the payments to particular patents. (A11154-55.) Because there was no evidence tying the Walt stock to a payment for patent rights, the stock's value was legally irrelevant, and the district court abused its discretion by admitting testimony that the Tufts rate should be doubled based on that stock value. *Uniloc,* 632 F.3d at 1318.

The Court should thus vacate the damages award and remand for a new trial without testimony about the Walt stock. When the patentee urges the jury to rely on a particular methodology to calculate damages, this Court's determination that the methodology should have been excluded requires a new trial. *See, e.g.*, *Uniloc*, 632 F.3d at 1321 (Fed. Cir. 2011) ("Even if the jury's damages calculation was not based wholly on the entire market value check, the award was supported in part by the faulty foundation of the entire market value…. [T]he fact that the entire market value was brought in as only a 'check' is of no moment."); *LaserDynamics, Inc. v. Quanta Computer, Inc.*, 694 F.3d 51, 81 (Fed. Cir. 2012) ("A new trial is required because the jury's verdict was based on an expert opinion that finds no support in the facts in the record."). It does not matter that Syntrix presented alternative reasons for doubling the Tufts rate. Syntrix supported the 6% rate "in part by the faulty foundation" of including the Walt stock, and that alone requires a retrial. *Id.* Moreover, if the Court

sets aside the jury's royalty rate for pre-verdict infringement, it should also vacate the ongoing royalty rate because the district court set the ongoing royalty by starting with the jury's rate and enhancing it. (A56.) Any change to the jury's rate would necessarily warrant reconsidering the ongoing royalty rate.

CONCLUSION

For the reasons above, the Court should reverse and remand with instructions for the district court to dismiss the suit, or, at a minimum, remand for further proceedings under the correct claim construction and free from error on damages.

Dated: March 12, 2014

Respectfully submitted,

/s/ Craig E. Countryman

Juanita R. Brooks
Craig E. Countryman
FISH & RICHARDSON P.C.
12390 El Camino Real
San Diego, CA 92130
Telephone: (858) 678-5070
Facsimile: (858) 678-5099

Adam Shartzer
Fish & Richardson P.C.
1425 K Street, NW, 11th Floor
Washington, DC 20005

Attorneys for Appellant,
Illumina, Inc.

ADDENDUM

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC.,

Plaintiff,

v.

ILLUMINA, INC.,

Defendant.

CASE NO. C10-5870 BHS

JUDGMENT IN A CIVIL ACTION

The Court has ordered that:

Plaintiff Syntrix Biosystems, Inc., recover from Defendant Illumina, Inc., 1) the total amount of one hundred fifteen million, one hundred six thousand, one hundred five dollars ($115,106,105), which is the verdict amount of ninety-five million, seven hundred ninety-five thousand, five hundred seven dollars ($95,795,507); prejudgment interest in the amount of seven million, two hundred thousand, four hundred dollars ($7,200,400) through June 5, 2013 plus fourteen days at the daily rate of five thousand, one hundred ninety-five dollars ($5,195), which is seventy two thousand, seven hundred thirty dollars ($72,730); and supplemental damages in the amount of twelve million, thirty-seven thousand, four hundred sixty-eight dollars ($12,037,468), and 2) an ongoing royalty at the rate of 8% per infringing sale.

This action was:

Tried by a jury with Judge Benjamin H. Settle presiding, and the jury has rendered a verdict.

Dated this 19th day of June, 2013.

Gretchen Craft, Courtroom Deputy

THE HONORABLE BENJAMIN SETTLE

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC.
a Delaware corporation

Plaintiff/Counter-Defendant

v.

ILLUMINA, INC.,
a Delaware Corporation

Defendant/Counter-Plaintiff

Case No. 3:10-cv-05870-BHS

AMENDED JUDGMENT IN A CIVIL ACTION

This Amended Judgment supersedes and replaces the Judgment entered on June 19, 2013 (Doc. No. 356).

The Court has ordered that:

1. Plaintiff Syntrix Biosystems, Inc., recover from Defendant Illumina, Inc., 1) the total amount of one hundred fifteen million, one hundred six thousand, one hundred five dollars ($115,106,105), which is the verdict amount of ninety-five million, seven hundred ninety-five

thousand, five hundred seven dollars ($95,795,507); prejudgment interest in the amount of seven million, two hundred thousand, four hundred dollars ($7,200,400) through June 5, 2013, plus fourteen days at the daily rate of five thousand, one hundred ninety-five dollars ($5,195), which is seventy two thousand, seven hundred thirty dollars ($72,730); and supplemental damages in the amount of twelve million, thirty-seven thousand, four hundred sixty-eight dollars ($12,037,468) ("Supplemental Damages"), and 2) an ongoing royalty at the rate of 8% per infringing sale.

2.a "Infringing Products" shall include (i) all BeadChip products (including BeadChip kits and BeadChips sold separately from kits) for which infringement was found and damages were assessed either at trial or included within the calculation of Supplemental Damages ("Existing BeadChip Products"), (ii) any BeadChip products (including BeadChip kits and BeadChips sold separately from kits) first sold on or after March 15, 2013 that are not colorably different from Existing BeadChip Products, (iii) any BeadChip product described in 2.a(i) and 2.a(ii) manufactured or assembled outside the United States if such BeadChip product is sold within or imported into the United States, and (iv) any BeadChip product described in 2.a(i) and 2.a(ii) that is manufactured or assembled outside the United States if Illumina's activities nevertheless constitute infringement pursuant to 35 U.S.C. § 271(f).

2.b "Infringing sales" shall mean sales of the "Infringing Products." If the Infringing Product sold is a BeadChip kit, the royalty shall apply to the entire price of the BeadChip kits.

3.a Illumina's obligation to pay damages assessed pursuant to trial proceedings and ongoing royalties as set forth in this Amended Judgment is stayed until the Court rules on the motion for stay of execution to be filed by Illumina under the Revised Agreed Proposed Briefing Schedule (Doc. No. 340).

3.b Illumina's obligation to pay ongoing royalties associated with this Amended Judgment is effective as of March 15, 2013 and expires upon the expiration of the '682 Patent.[1] Ongoing royalty payments made under this Amended Judgment shall be escrowed either (a) with this Court until this Amended Judgment becomes final and not subject to further appeal, in which case the escrowed funds and any interest on such escrowed funds will be released to the prevailing party, or (b) pursuant to other arrangements to which the parties may agree. If this Amended Judgment becomes final and not subject to further appeal, Illumina shall make ongoing royalty payments directly to Syntrix.

4. Royalties shall be paid on all sales based on shipments during the fiscal quarter and shall be accompanied by an accounting of all Infringing Product sales that were shipped during the fiscal quarter. Royalty payments shall be made no later than the 30th day following the close of the fiscal quarter. Royalty payments for any fiscal quarters ending before the entry date of this Amended Judgment shall be made within 30 days of the entry of this Amended Judgment. Thereafter, royalty payments shall be made within 30 days of the end of the subsequent fiscal quarters. If the 30th day following the end of a fiscal quarter or the entry of this Amended Judgment is a Saturday, Sunday, or holiday, then the payment is due on the next business day.

5. The first royalty payment should also include all royalties for all sales of Infringing Products that were previously not accounted for in the damages assessment presented at trial and in the supplemental damages awarded by the Court. Notwithstanding the previous sentence, royalties

[1] This obligation is subject to any superseding court order issued after the date of this Amended Judgment by this Court on any post-judgment motion or by an appellate court on appeal.

shall not be assessed on any shipments resulting from any orders upon which damages were assessed at trial or included within the calculation of supplemental damages.

6. Illumina shall keep complete, true, and accurate books of account and records, including, but not limited to, units and dollar amounts of sales for Infringing Products, for the purpose of determining the royalty amounts payable. Illumina shall certify compliance with this Amended Judgment annually 60 days after the close of its fiscal year and ensure that the quarterly royalty payments for the preceding fiscal year are in accordance with this Amended Judgment. Such certification shall be accompanied with any underpayments for the preceding four fiscal quarters. If Illumina's certification process reveals an underpayment in excess of five percent (5%) of the amount owed over the course of Illumina's fiscal year, Illumina shall pay interest on the unpaid royalties at a rate equal to the mid-point between the 3-month Treasury bill rate and the prime rate, compounded quarterly.

7. Syntrix shall have the right, upon reasonable notice and at its expense, to direct an independent accounting firm to inspect and audit the relevant accounting and sales books and records, including but not limited to, the units and dollar amounts of sales for each Infringing Product. The audit may be made once per year and may cover any period, after March 15, 2013, within the previous four completed fiscal years prior to the audit, provided that such period has not been previously audited. Illumina shall have the right to examine the results, findings, and supporting data from the audit. In the event an audit reveals an underpayment, Illumina shall remit payment of such amount to Syntrix within thirty (30) days of receiving written notice of such underpayment. In addition, if any such audit reveals an underpayment in excess of five percent (5%) of the amount owed for the period audited, Illumina shall pay the reasonable fees and expenses

actually incurred relating to the audit as well as interest on the unpaid royalties at a rate equal to the mid-point between the 3-month Treasury bill rate and the prime rate, compounded quarterly, which Illumina shall pay to Syntrix within thirty (30) days of notice from Syntrix to Illumina. If the audit discloses an overpayment, Syntrix shall credit Illumina the overpayment amount for future payments. If the overpayment exceeds the royalty amount owed by Illumina at the expiration of this Amended Judgment, Syntrix shall refund Illumina the amount of overpayment in excess of Illumina's owed royalties within thirty (30) days.

8. Syntrix and Illumina shall meet and confer in good faith in an attempt to resolve all disputes that may arise under this Amended Judgment. The Court specifically retains jurisdiction to enforce, modify, or terminate its Amended Judgment as the equities may require, and to adopt procedures for resolution of any dispute under this Amended Judgment.

9. These terms are binding on Syntrix and Illumina, as well as their successors or assignees.

10. All notices of any asserted breach or any other asserted dispute under the terms of this Amended Judgment shall be in writing and shall be deemed given when sent by (a) prepaid, registered or certified mail, addressed to the party at the notification address provided by each party, or (b) by private courier, service signature for delivery required, addressed to the party at the notification address provided by each party. Each party shall provide its notification address within seven (7) days of the entry of this Amended Judgment. Each party may change such notification address from time to time by notice so given.

11. The damages awarded in this Amended Judgment shall accrue post-judgment interest pursuant to 28 U.S.C. § 1961 from the date the original Judgment was entered on June 19, 2013.

This action was:

Tried by a jury with Judge Benjamin H. Settle presiding, and the jury has rendered a verdict.

DATED this 1 day of July, 2013.

HONORABLE BENJAMIN H. SETTLE
United States District Judge

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC., a Delaware corporation,

Plaintiff,

v.

ILLUMINA, INC., a Delaware corporation,

Defendant.

CASE NO. 10-5870BHS

ORDER CONSTRUING CLAIMS

This matter comes before the Court pursuant to *Markman v. Westview Instruments, Inc.*, 52 F.3d 967 (Fed. Cir. 1995), to construe the disputed claim terms of United States Patent No. 6,951,682 ("the '682 Patent"). The Court has reviewed each party's opening and responsive brief, heard oral argument of counsel, and considered the remainder of the file and hereby construes the claim terms at issue as stated herein.

I. PROCEDURAL HISTORY

On November 24, 2010, Plaintiff Syntrix Biosystems, Inc. ("Syntrix") filed a complaint against Defendant Illumina, Inc. ("Illumina") asserting numerous causes of action, including infringement of the '682 Patent. Dkt. 1.

On January 24, 2012, both parties filed opening claim construction briefs. Dkts. 55 & 57. On February 8, 2012, both parties responded. Dkts. 60 & 61. On May 7, 2012, the Court held a technology tutorial and claim construction hearing. *See* Dkt. 163.

II. PATENT

On October 4, 2005, the United States Patent and Trademark Office ("USPTO") issued the '682 Patent, titled "Porous Coatings Bearing Ligand Arrays and Use Thereof," to John A. Zebala ("Zebala"). Under the "Technical Field" heading of the specification, the patent provides as follows:

> The present invention relates generally to articles comprising porous coatings, and to methods for preparing and using such articles. The invention is more particularly related to methods for fabricating articles having patterned porous coatings, which may be used, for example, to screen large numbers of discrete compounds for diagnostic or drug discovery purposes.

'682 Patent, col. 1, ll. 11–17. The specification also contains a glossary that provides the definitions of certain terms. *Id.*, col. 7, l. 23 to col. 22, l. 12 ("Glossary").

On June 11, 2008, Illumina filed a Request for Ex Parte Reexamination of claims 1-7, 9, 10, 15, 16, 19–23, 28–33, 37–40, 71–75, 78, 80, 81, 83–85, 87, 125–130, 154 and 155. Dkt. 56, Declaration of Erick S. Ottoson ("Ottoson Decl."), Exh. 3 at 5. On August 10, 2008, the USPTO issued an order for reexamination. *Id.* On June 29, 2010, the USPTO issued a reexamination certificate. Dkt. 58, Declaration of Christopher Schenck ("Schenck Decl."), Exh. A at 63-65. As a result of reexamination, the USPTO (1) confirmed the patentability of claims 1-7, 9, 10, 15, 16, 19-23, 15, 28-33, 37-40, 154 and 155; (2) determined that claims 71 and 125 were patentable as amended; and (3)

determined that claims 72-75, 78, 80, 81, 83-85, 87 and 126-130 were patentable as dependent upon an amended claim. *Id.*

III. DISCUSSION

A. Legal Standard

It is the obligation of the court to construe as a matter of law the meaning of language used in a patent claim. *Markman*, 52 F.3d at 979. In construing a patent's claim terms, a court must consider the intrinsic evidence in the record. *See Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005). Intrinsic evidence includes the ordinary and customary meaning of the claim terms, the specification of the patent, and the patent's prosecution history. *Id.*

The ordinary and customary meaning of a term is defined by a person of ordinary skill in the art at the time of the invention. *Id.* The context in which a term is used can be "highly instructive" in resolving the meaning of the term. *Id.* at 1314. For example, if a claim has the term "steel baffle," it strongly implies that the term "baffle" does not inherently include objects made of steel. *Id.* Other claims in a patent may also provide valuable contextual cues for deciphering the meaning of a term. *Id.* If a limitation is present in a dependent claim, then there is a presumption that the limitation is not present in the parent claim. *Id.* at 1314–15.

The claims must also be read in light of the specification. *See Markman*, 52 F.3d at 979. The specification is always highly relevant to the meaning of a claim term: "Usually, it is dispositive; it is the single best guide to the meaning of a disputed term." *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1582 (Fed. Cir. 1996). If the

specification reveals a definition of a claim term that is different from how that term would otherwise be used, then "the inventor's lexicography governs." *See Phillips*, 415 F.3d at 1316. Courts should take care, however, not to import limitations from the specification into the claims. *Id.* at 1323. For example, even if the specification describes very specific embodiments, the claim terms should not be confined to those embodiments. *Id.*

The prosecution history of a patent is the last piece of intrinsic evidence that a court should consider when construing the claims of the patent. *Id.* at 1317. The prosecution history provides evidence of how the U.S. Patent and Trademark Office ("PTO") and the inventor understood the patent. *Id.* A court, however, should be aware that the prosecution history represents the ongoing negotiation between the PTO and the applicant, rather than the final product. *Id.* As such, the prosecution history may lack the clarity of the specification and may not be as useful for claim construction purposes. *Id.* In certain instances, the prosecution history may provide guidance of an applicant's intent to specifically limit the scope of a given claim term. *Id.* For example,

> where the patentee has unequivocally disavowed a certain meaning to obtain his patent, the doctrine of prosecution disclaimer attaches and narrows the ordinary meaning of the claim congruent with the scope of the surrender.

Omega Eng'g, Inc. v. Raytek Corp., 334 F.3d 1314, 1324 (Fed. Cir. 2003). The Federal Circuit, however, has "declined to apply the doctrine of prosecution disclaimer where the alleged disavowal of claim scope is ambiguous." *Id.*; *Rexnord Corp. v. Laitram Corp.*,

274 F.3d 1336, 1347 (Fed. Cir. 2001) (refusing to limit the ordinary meaning of the claim because the alleged disclaimer in the file wrapper was at best "inconclusive").

Extrinsic evidence is the last category of evidence a court may consider when construing patent claims. *Id.* Such extrinsic evidence includes expert and inventor testimony, dictionaries, and learned treatises. *Id.* On its own, extrinsic evidence is unlikely to be reliable in guiding the court's claim construction. *Id.* at 1319. Instead, extrinsic evidence should be considered in the context of the intrinsic evidence. *Id.* A court may also use extrinsic evidence to determine how a person of ordinary skill in the art would understand the claimed invention. *Id.*

Although it is the court's duty to resolve fundamental disputes among the parties as to the scope of a claim term, it is not the court's duty to construe every claim term, or to repeat or restate every claim term. *See U.S. Surgical Corp. v. Ethicon, Inc.*, 103 F.3d 1554, 1568 (Fed. Cir. 1997); *02 Micro Int'l Ltd. v. Beyond Innovation Tech Corp.*, 521 F.3d 1351, 1362 (Fed. Cir. 2008).

> Ultimately, the interpretation to be given a term can only be determined and confirmed with a full understanding of what the inventors actually invented and intended to envelop with the claim. The construction that stays true to the claim language and most naturally aligns with the patent's description of the invention will be, in the end, the correct construction.

Phillips, 415 F. 3d at 1312 (citing *Renishaw PLC v. Marposs Societa' per Azioni*, 158 F.3d 1243, 1250 (Fed. Cir. 1998).

With these standards and rules in mind, the Court turns to the undisputed and disputed claim terms of the patents in question.

B. Undisputed Terms

The parties request that the Court adopt the agreed construction of twelve claim terms. Dkt. 54 at 1-2. Some of these proposed constructions are almost identical to the definitions set forth in the Glossary. The Court finds that adopting preexisting definitions is an exercise in redundancy. *U.S. Surgical Corp. v. Ethicon, Inc.*, 103 F.3d 1554, 1568 (Fed. Cir. 1997) (Claim construction "is not an obligatory exercise in redundancy.") Moreover, the parties request that the Court adopt the construction that certain terms carry their "[o]rdinary meaning in the art." *See* Dkt. 54 at 1–2 (agreed construction of "detached"). This is the construction for every undisputed, undefined term (*Phillips*, 415 F.3d at 1313) and there is no reason to emphasize some terms over others. Therefore, the Court declines to adopt a majority of the agreed constructions.

With regard to the terms that are not defined in the Glossary and do not carry their ordinary meaning, the Court adopts the following constructions:

base labile moiety	A portion of a molecule that is cleaved upon exposure to a particular basic chemical or pH.
continuous / continuous porous coating	A "porous coating" is said to be "continuous" when the coating covers the surface of the substrate with virtually no discontinuities or gaps.
substantially uniform thickness	"Substantially uniform thickness" is when the thickness of the coating varies by no more than 30% over the entire coated area.

C. Disputed Terms

The parties dispute the construction of eight terms in the '682 Patent as the terms are used in independent claim 1 and independent claim 21. Dkt. 54 at 3-9.

1. "coated article"

In this case, the parties have failed to show that there is an actual dispute as to this term. Although neither party addressed the issue during the *Markman* hearing, it appears that the dispute was what the coated article "comprised." Dkt. 54 at 3. In patent law, the "word 'comprising' transitioning from the preamble to the body signals that the entire claim is presumptively open-ended." *Gillette Co. v. Energizer Holdings, Inc.*, 405 F.3d 1367, 1371 (Fed. Cir. 2005) (citing *Crystal Semiconductor Corp. v. TriTech Microelectronics Int'l, Inc.*, 246 F.3d 1336, 1347 (Fed. Cir. 2001)). Neither party has cited any intrinsic evidence that negates the application of this well settled presumption for the claims of the '682 Patent. Therefore, the Court declines to construe "coated article."

2. "substrate"

The parties propose constructions for the term "substrate" as follows:

Syntrix's Proposed Construction	A substrate forms a rigid support on which to support the porous coating
Illumina's Proposed Construction	The support on which a porous coating is attached, which may be prepared from essentially any material, may have any convenient shape, and is preferably flat but may have a variety of alternative surface configurations, including raised and/or depressed regions

Both constructions are distilled from the same paragraph in the specification, which provides as follows:

> A. Substrate Selection and Preparation
>
> Nearly any conceivable substrate may be employed, including substrates that are biological, nonbiological, organic, inorganic or a

> combination of any of these. The substrate may have any convenient shape, such as a disc, square, sphere, circle, or any other suitable shape, and may be formed, for example, as a particle, strand, precipitate, gel, sheet, tube, sphere, container, capillary, pad, slice, film, plate or slide. The substrate should form a rigid support on which to support the porous coatings described herein, and is preferably flat, although it may have a variety of alternative surface configurations, including raised and/or depressed regions. The substrate may be prepared from essentially any material. . . .

'682 Patent, col. 22, l. 61 to col. 23, l. 7.

With regard to Illumina's construction, it imports a limitation that a porous coating is "attached" to the supporting substrate. The intrinsic evidence, however, clearly states that attachment is optional. *See* '682 Patent, col. 23, ll. 28–30 ("Optionally, a surface of a substrate may have an adhesive layer, which is stably attached to the substrate and promotes adhesion of the porous coating to the substrate.") The Court declines convert this optional embodiment into a claim limitation.

Syntrix's proposed construction also converts an optional embodiment into a claim limitation by construing the word "should" as a mandatory limitation that the substrate "forms a rigid support." The Court also declines to adopt the proposed conversion.

Rejecting both proposed construction, the Court finds that the ordinary meaning of the term substrate is adequately provided by the specification. Moreover, each party may argue to the jury the limitations that are more important to the respective positions, but the specification provides the best evidence of what the inventor "intended to envelop with the claim . . . ." *Phillips*, 415 F. 3d at 1312.

3. "porous coating"

The parties propose constructions for the term "porous coating" as follows:

Syntrix's Proposed Construction	A coating that is rigid, does not swell, and contains void regions ranging from 1 to 1500 nm in diameter resulting in porosities ranging from 0.15 to 0.99, where porosity is defined as the fraction of the coating volume which has pores.
Illumina's Proposed Construction	A coating is "porous" if it contains void regions ranging from 1 to 1500 nm in diameter resulting in porosities ranging from 0.15 to 0.99, where porosity is defined as the fraction of the coating volume which has pores, i.e., the fraction of the volume of the packed particles that is void space. A porous coating must be porous to aqueous media.

"In an effort to come to agreement on this claim term," Syntrix altered its initial proposed construction. Dkt. 61 at 6. Although the parties' proposed constructions are similar in some respects, the parties dispute whether three limitations should be added to the definition of "porous coating": (1) Syntrix requests the limitation that the porous coating "is rigid, does not swell"; (2) Illumina requests the limitation that "the fraction of the volume of the packed particles that is void space"; and (3) Illumina requests the limitation that a "porous coating must be porous to aqueous media."

First, the specification supports Syntrix's proposed limitation that the porous coatings are rigid and do not swell. For example, the inventor stated that "[r]igid porous supports that do not require swelling . . . offer the potential to increase ligand surface density . . . ." '628 Patent, col. 2, ll. 25–27. The inventor also stated that "[p]orous coatings provided herein . . . . do not swell or distort substantially . . . ." *Id.*, col. 7, ll. 7–9. Illumina, however, argues that it would be improper to import these limitations because the limitations are not in the glossary definition that explains when a coating is porous. Dkt. 60 at 3–4. Although a term should usually be construed as provided in the

Glossary, for this term, the inventor used limiting language such as "provided herein." This language supports Syntrix's position that the limitations are included in the construction of the term. Therefore, the Court adopts this portion of Syntrix's proposed construction.

Second, Illumina proposes the limitation that "the fraction of the volume of the packed particles that is void space." Although Illumina concedes that this limitation appears in the Glossary definition for "particles," it argues that the inventor provided two definitions for the term "porosity" and the two definitions are based on the concept of packing particles together. Dkt. 55 at 12–13. Illumina concludes that the Court should construe "fraction of the volume of the packed particles that is void space" as a rephrasing or clarification of the phrase "fraction of the coating volume which has pores." *Id.* Syntrix counters that this proposed construction limits "the coating volume" to a "volume of packed particles." Dkt. 61 at 7–8. Syntrix argues that this limitation is improper because the inventor disclosed coating volumes of particles that are linked with or without binders. *Id.*; '628 Patent, col. 11, ll. 22–26. The Court agrees with Syntrix because the intrinsic evidence supports the contention that a coating volume is not necessarily a volume of packed particles. Therefore, the Court declines to adopt Illumina's proposed limitation.

Third, Illumina proposes the limitation that a "porous coating must be porous to aqueous media." Illumina contends that, during the reexamination proceeding, the inventor "disclaimed any construction of 'porous coating' that would encompass coatings that are not porous to aqueous media." Dkt. 55 at 14. The intrinsic evidence, however,

does not support Illumina's contention. The record on reexamination shows that the patentee argued that the Examiner's interpretation of the prior art[1] was incorrect and not that the '628 Patent included limitations that overcame the prior art. *See* Schenck Decl., Exh. 5, ¶ 5. Specifically, Syntrix's expert declared as follows:

> I do not see where the Walt reference coating is porous because the Walt reference exemplifies its coating with Nafion that the Walt reference admits is not porous to aqueous media (the only relevant medium for such arrays is an aqueous medium) on col. 12 lines 65–67.

Id. At the very least, this statement is ambiguous as to the limitations of the '628 Patent and does not show that the "patentee has unequivocally disavowed" the scope of the claim requiring the proposed limitation. *Omega*, 334 F.3d at 1324. Therefore, the Court declines to adopt Illumina's proposed limitation.

Based on the foregoing analysis, the Court construes the term "porous coating" as proposed by Syntrix, which is as follows:

"porous coating"	A coating that is rigid, does not swell, and contains void regions ranging from 1 to 1500 nm in diameter resulting in porosities ranging from 0.15 to 0.99, where porosity is defined as the fraction of the coating volume which has pores.

4. "gelled network"

The parties propose constructions for the term "gelled network" as follows:

Syntrix's Proposed Construction	A "gelled network" refers to an aggregation of particles linked together to form a porous three-dimensional network. Particles may be linked covalently or noncovalently through the use of a polymeric binder.

[1] The relevant prior art on this issue is U.S. Patent 6,023,540 issued to David R. Walt and Karri Lynn Michael (the "Walt reference").

	Alternatively, particles may be linked covalently or noncovalently without the use of a binder, through interactions of chemical groups on the surface of the particles. Noncovalent interactions that may be employed in polymeric binders or surface groups include, for example, electrostatic interactions, hydrogen bonding, metal coordination, and Van der Waals interactions. The extent of linking sufficient to constitute a "gelled network" will be such that less than 20%, and more preferably less than 5%, of the network is lost after contact with any process agent.
Illumina's Proposed Construction	"Gelled network" refers to an aggregation of particles linked together to form a porous three-dimensional network. The requisite "three-dimensional network" is not satisfied by a planar, two-dimensional configuration of particles.

Syntrix concedes that its proposed construction "merely quotes the specification, almost verbatim." Dkt. 57 at 14. Adopting language verbatim from the specification is an exercise in redundancy that the Court declines to do.

With regard to Illumina's proposed construction, it proposes two sentences. The first sentence is the introductory sentence for this term in the Glossary. The patentee provided a complete definition in the Glossary and the Court declines to shorten that definition as a matter of law.

On the other hand, Illumina's second sentence is based on the proposition that the term "gelled network" does not encompass a network that consist of a monolayer of particles, which is a concept that is aptly described by the phrase "tomatoes on a table." Illumina argues that this limitation is supported by the specification and the patentee's statements during reexamination. Dkts. 55 at 14–17 & 60 at 6–10. First, the Glossary definition provides as follows: "Gelled network" refers to an aggregation of particles

linked together to form a porous three-dimensional network. '628 Patent, col. 11, ll. 20–21. Illumina argues that the adjective "three-dimensional" describes the network of linked particles and not the physical dimensions of the particles themselves. Dkt. 60 at 6. Illumina's argument is supported by a preferred embodiment ('628 Patent, Fig. 1A) and the fact that the smallest disclosed coating thickness, 1.0 µm (*id.*, col. 28, ll. 1–2), is ten times the diameter of the largest disclosed particle, 1000 Å or 0.1 µm (*Id.*, col. 27, ll. 60–62). However, claim terms should not be limited to disclosed embodiments, *Phillips*, 415 F.3d at 1323. Moreover, there is no clear indication that the patentee intended to encompass networks that included only two or more layers of particles. Without such a clear exclusion, the Court is unable to conclude that a person of ordinary skill in the art would interpret a "three dimensional network" to exclude a monolayer of particles.

Second, Illumina argues that Syntrix disavowed this broad claim scope during reexamination. Dkt. 55 at 15–17. During reexamination, the examiner rejected claim 1 in light of multiple pieces of prior art, two of which were Goldberg[2] and Suel[3]. *See* Schenck Decl., Exh. B. Syntrix responded with numerous arguments distinguishing various features of the claimed invention from the prior art. *Id.*, Exh. D. Illumina has selectively plucked portions of this response to support an argument that Syntrix expressly disavowed a planar, two-dimensional configuration of particles. Dkt. 55 at 15–16. However, Syntrix's reexamination arguments were organized on a claim-by-claim basis. Schenck Decl., Exh. 6. With regard to claim 1, Syntrix did not state that claim 1

---

[2] WO 97/39151 published October23, 1997.
[3] WO 97/40385 published October 30, 1997.

overcame the prior art because the gelled network of particles consisted of two or more layers of particles. In fact, Syntrix argued that the Suel "disclosure does not disclose (actually or inherently) the required monolayer of claim 1." *Id.* at 10–11, ¶ 5. Therefore, the Court concludes that Syntrix did not unequivocally disavow a monolayer embodiment during reexamination.

Based on the foregoing analysis, the Court declines to adopt either party's proposed construction and the term shall be construed as set forth in the Glossary.

5. "particles"

The parties propose constructions for the term "particles" as follows:

Syntrix's Proposed Construction	Particles are discrete objects that when packed together yield a porosity ranging from 0.15 to 0.99, where porosity is defined as the fraction of the volume of the packed objects that is void space. Particles may have any shape, and may be, for example, spheres, cubes or irregularly shaped objects.
Illumina's Proposed Construction	Particles are discrete objects, having an average size of no greater than 1000 Å, that when packed together yield a porosity ranging from 0.15 to 0.99, where porosity is defined as the fraction of the volume of the packed objects that is void space. Particles may have any shape, and may be, for example, spheres, cubes or irregularly shaped objects.

The only dispute regarding this term is Illumina's proposal to add the limitation that particles are discrete objects "having an average size of no greater than 1000 Å." Illumina asserts that this limitation "is mandated by the specification's clear disclaimer of larger particles . . . ." Dkt. 55 at 19. In certain cases, "the specification may reveal an intentional disclaimer, or disavowal, of claim scope by the inventor." *Phillips*, 415 F.3d at 1316. In such cases, the Court should interpret the claim more narrowly than it

otherwise would to give effect to the inventor's intent to disavow a broader claim scope. *Id.*; *Honeywell Int'l, Inc. v. ITT Indus., Inc.,* 452 F.3d 1312, 1319-20 (Fed. Cir. 2006); *SciMed Life Sys., Inc. v. Advanced Cardiovascular Sys., Inc.*, 242 F.3d 1337, 1342-44 (Fed. Cir. 2001). For example, the *Honeywell* court stated that where

> the written description has gone beyond expressing the patentee's reference for one material over another . . . repeated derogatory statements concerning one type of material are the equivalent of disavowal of that subject matter from the scope of the patent's claims.

452 F.3d at 1318.

In this case, the relevant portion of the specification provides that "a primary particle size greater than 1000 Å yields porous coatings with surface areas too small to be useful in the present invention." '628 Patent, col. 27, ll. 48–50. The Court finds that the phrase "too small to be useful" is neither a disavowal of subject matter nor an equivalent thereof. This finding is supported by a separate portion of the specification that provides "the particles have a primary particle size of less than 2000 Å, and more preferably less than 1000 Å and still more preferably less than 500 Å." '628 Patent, col. 24, ll. 52–56. Therefore, the Court rejects Illumina's proposed limitation and adopts the agreed upon remainder of the proposed constructions, which is as follows:

"particles"	Particles are discrete objects that when packed together yield a porosity ranging from 0.15 to 0.99, where porosity is defined as the fraction of the volume of the packed objects that is void space. Particles may have any shape, and may be, for example, spheres, cubes or irregularly shaped objects.

6. "attached"

The parties propose constructions for the term "attached" as follows:

Syntrix's Proposed Construction	Attachment of compounds to the porous coating may be covalent or via adsorption, with or without the use of a linker.
Illumina's Proposed Construction	A compound is said to be "attached" to a porous coating if the compound substantially remains on the surface during photoresist application and removal (i.e., at least 60% of the attached compounds are not removed when such processes are performed as described herein). Attachment may be covalent or noncovalent.

The context in which the term "attached" is used is "highly instructive" in resolving the parties' dispute. *Phillips*, 415 F.3d at 1313. Claim 1 provides, in part, as follows: "wherein the porous coating has two different compounds attached thereto . . . ." The specification provides, as Syntrix proposes, that "[a]ttachment of compounds to the porous coating may be covalent or via adsorption, with or without the use of a linker." '682 Patent, col. 3, ll. 45–47. On the other hand, Illumina cites a portion of the specification that describes compounds being attached to a "substrate." *Id.*, col. 8., ll. 44–57 (Illumina substituted "porous coating" for "substrate" in its proposed construction). Therefore, the Court adopts Syntrix's proposed construction because the term "attached" in used in the context of placing the compounds with the porous coating, not the substrate. The Court construes the term "attached" as follows:

"attached"	Attachment of compounds to the porous coating may be covalent or via adsorption, with or without the use of a linker.

7. "wherein the compounds are attached at known discrete full thickness volumes"

The parties propose constructions for the phrase "wherein the compounds are attached at known discrete full thickness volumes" as follows:

Syntrix's Proposed Construction	"attached" – see definition above (i.e., attachment of compounds to the porous coating may be covalent or via absorption, with or without the use of a linker. '682 Patent, col. 3 ll. 45–47). "at" – plain and ordinary meaning – a point or part of the "known discrete full thickness volumes". - "discrete" – plain and ordinary meaning – generally constituting a separate entity; individually distinct. "full thickness volume" refers to the volume of a coating region as defined by the boundaries of the surface plane, the base plane (i.e., the plane of the coating in contact with the substrate surface or the surface of an adhesive layer), and the region. For example, a rectangular region of dimensions l and w on a coating of thickness t will have a full thickness volume of 1xwxt. A circular region of radius r will have a full thickness volume of r^2t on the same coating.
Illumina's Proposed Construction	"Attached at" "known discrete full thickness volume" ("KDFTV") means that compounds are attached throughout the entire volume of the KDFTV. - A KDFTV refers to the volume of a coating region as defined by the boundaries of the surface plane, the base plane, and a localized area of the substrate surface on which a substantially pure group of compounds is, was, or is intended to be attached. Such regions do not overlap. In addition, the "volume" in KDFTV is a three-dimensional notion that excludes planar surfaces and coatings (which are two-dimensional).

Illumina's proposed construction imports three distinct limitations. First, Illumina proposes that the term be construed to exclude a monolayer configuration. The Court declines to adopt this limitation based on the analysis of the term "gelled network of particles."

Second, Illumina proposes the limitation that a volume consists of "a localized area of the substrate surface on which a substantially pure group of compounds is, was, or is intended to be attached. Such regions do not overlap." This is the Glossary definition for the term "discrete known region." '628 Patent, col. 10, ll. 39–41. Syntrix argues that, based on the canon of claim differentiation, it would be improper to import the definition of "discrete known region" into the term "known discrete" of claim 1 because "discrete known region" appears in claims 41, 69, 71, 121, and 154. Dkt. 61 at 13. In fact, both "discrete known region" and "known discrete full thickness volumes" are used in claim 41, which supports Syntrix's position that the terms are not identical. The Court agrees and concludes that one of ordinary skill in the art would understand that the Glossary definition of "discrete known region" is only applicable to that term as it is used in the other claims. Moreover, it is clear that the patentee acted as his own lexicographer as to the term "discrete known region" and not to the term "known discrete." Therefore, the term "known discrete" carries its ordinary and customary meaning.

Third, based on arguments Syntrix made during reexamination, Illumina proposes the limitation "that compounds are attached throughout the entire volume." In the specification, the patentee disclosed that compounds and ligands are attached to the porous coating at separate "full-thickness volumes." '628 Patent, col. 37, ll. 22–23 ("Compounds may be attached to a porous coating within separate full-thickness volumes") & ll. 35–37 ("In preferred embodiments, a coated article comprises an array of ligand groups attached to the porous coating within separate full-thickness volumes.").

Thus, if the porous coating was the field of play, the separate "full-thickness volumes" would be the positions of the individual players located across the field.

During the reexamination proceeding, the examiner rejected claim 1 as anticipated by Goldberg because, in part, "Goldberg still further discloses that the arrays are generated by photolithography . . ., [which] allows for the compounds to be attached at known locations in the porous coating." Schenck Decl., Exh. B at 9, ¶ 6. Syntrix responded and submitted the declarations of Dr. Roger Bumgarner and Dr. Kilian Dill. Dr. Bumgarner declared, in part, as follows:

> The Zebala disclosure teaches me that compounds are attached on the surface of the gelled network of particles throughout the volume of a coating, as also stated in the claim element: "the compounds are attached at known discrete full thickness volumes". Although the teaching in Goldberg at page 18, lines 3-6 discloses arrays generated by photolithgraphy, the Goldberg disclosure provides no evidence of the Zebala claim element that "the compounds are attached at known discrete full thickness volumes."

Id., Exh. F, ¶ 12. Based on this declaration, Syntrix responded to the rejection by arguing that:

> Goldberg page l8 lines 3-6 does not disclose the notion of "the compounds to be attached at known locations in the porous coating" because the sentence refers to miniaturization, not known locations ("Since photolithography is used, the process can be readily miniaturized to generate high density arrays of oligonucleotide probes."). Therefore, [the specific limitation] is not supported by the referenced Goldberg section or anywhere else in Goldberg.

Id., Exh. D at 5–6, ¶ 5.

The examiner also rejected claim 1 as being anticipated by Walt. With regard to the instant term, the examiner provided, in part, as follows:

> Walt further discloses that the beads of the porous coating have different chemical functionalities (i.e., two or more different functional compounds) attached to them and that the beads having "different compounds" are attached at discrete locations. Furthermore, the beads are distinguished using microscopy analysis, and the optical signature of the array may be decoded such that the discrete location of each bead is known (See column 3, lines 17-24; column 4, lines 20-27; and column 15, lines 14-16) (two or more different compounds attached to the porous coating, attached at known discrete full thickness volumes).

Id., Exh. B at 15, ¶ 8. Dr. Dill declared, in part, as follows:

> The Walt microspheres are derivatized with different chemical functionalities. Yet the Walt overlying polymer is not. Therefore, as I read Walt, using terminology from Zebala, the chemical functionalities are attached at only a *partial thickness* of the total (non-porous) coating (that is, relative to substrate, the (non-porous) coating is taller than the maximum height of the chemical functionalities).

Id., Exh. C, ¶ 6 (emphasis in original). Based on his declaration, Syntrix responded by arguing that:

> The Dill Declaration Section 6 provides that the non-porous coating in Walt is only a "partial thickness." As such, the Walt partial thickness (non-porous) coating cannot be substantially uniform in thickness due to the coating being only partial thickness. Therefore, Walt does not disclose the "substantially uniform thickness" element of claims 1, 71 and 154. With regard to the gelled network of particles element, this is tied to a porous coating, yet Walt (as described above) does not disclose a porous coating (only a non-porous one) so the gelled network of particles is not anticipated by Walt due to the coating in Walt being both partial thickness and non-porous.

Id., Exh. D at 15, ¶ 2.

Illumina argues that, based on statements made in the declarations submitted in support of its response, "Syntrix cannot now secure a claim construction in which compounds need not be attached 'throughout the volume of a coating' and may be attached at only a 'partial thickness' of the total coating." Dkt. 55 at 18-19. However,

the reexamination record does not support an unequivocal disavowal of this claim scope. In fact, it appears that the declarants were concerned with issues entirely separate from the issue of whether a compound must be attached throughout the entire thickness of the coating. For example, Dr. Kill opined that Walt disclosed a coating that consists of separate layers of which only some of the layers provided the proper chemical functionalities. Syntrix responded that the patentee disclosed a continuous porous coating wherein the entire thickness provides the proper chemical functionalities. Moreover, Dr. Bumgarner's use of the term "throughout" was in relation to the coating and not in relation to the "known discrete full thickness volumes." At the very least, his statement is ambiguous as to whether compounds are attached throughout the surface area of the coating, the field of play, or throughout the thickness of the coating. Syntrix's response on this point is also instructive because it states that Goldberg's "sentence refers to miniaturization, not known locations." Therefore, the Court declines to adopt Illumina's proposed construction because Syntrix did not unequivocally disavow the challenged claim scope on reexamination.

With regard to Syntrix's proposed construction, it involves the plain and ordinary meaning of terms as well as definitions set forth in the Glossary. The Court declines to adopt the proposed construction for "full thickness volumes" because the term is adequately defined in the Glossary. However, the Court will adopt Syntrix's proposed constructions for the terms "discrete" and "at" because the proposed constructions are based on the plain and ordinary meaning of the terms. Therefore, the Court construes these terms as follows:

"at"	A point or part of the "known discrete full thickness volumes"
"discrete"	generally constituting a separate entity; individually distinct

8. "linker"

The parties propose constructions for the term "linker" as follows:

Syntrix's Proposed Construction	A linker may serve a variety of functions, including spacing attached compounds from the surface, facilitating receptor recognition of attached ligands, or supplying a labile linkage that allows ligands to be detached from the surface. A spacer is a small molecule that serves to separate the synthesized compound from the surface. Spacers may be used alone, or incorporated into linkers.
Illumina's Proposed Construction	A linker is a molecule that physically attaches a compound to a porous coating. Linkers may serve the functions of spacing attached compounds from the surface, facilitating receptor recognition of attached ligands, or supplying a labile linkage that allows ligands to be detached from the surface.

Syntrix's proposed construction is pulled from the specification. '628 Patent, col. 35, ll. 21–27. This construction, however, incorporates the definition of a spacer, which appears to be a separate entity. For example, the specification clearly states that "[s]pacers may be used alone, or incorporated into linkers." '628 Patent, col. 35, ll. 26–27. Therefore, the Court declines to incorporate the definition of spacers into the construction of linkers.

Illumina agrees with the remainder of Syntrix's construction, but it proposes the additional limitation that "[a] linker is a molecule that physically attaches a compound to a porous coating." Syntrix offers numerous reasons why this limitation is improper. Dkt.

57 at 27. For example, the patentee indicated that ligands can be attached covalently or non-covalently to a surface by use of a linker. '628 Patent, col. 12, ll. 58–60. Additionally, claim 54 provides that a "compound is attached to the substrate by a linker." If a limitation is present in dependent claim 54, then there is a presumption that the limitation is not present in the parent claim 21. *See Phillips*, 415 F.3d at 1314–15. There is no evidence to overcome this presumption. Therefore, the Court declines to adopt Illumina's proposed limitation and construes the term "linker" as follows:

"linker"	A linker may serve a variety of functions, including spacing attached compounds from the surface, facilitating receptor recognition of attached ligands, or supplying a labile linkage that allows ligands to be detached from the surface.

IV. ORDER

Therefore, it is hereby **ORDERED** that the claims of the '628 Patent are construed as provided herein.

Dated this 11th day of June, 2012.

BENJAMIN H. SETTLE
United States District Judge

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC.,

Plaintiff,

v.

ILLUMINA, INC.,

Defendant.

CASE NO. C10-5870 BHS

ORDER DENYING DEFENDANT'S MOTION FOR JUDGMENT AS A MATTER OF LAW OR NEW TRIAL

This matter comes before the Court on Defendant Illumina, Inc.'s ("Illumina") motion for judgment as a matter of law or new trial (Dkt. 363). The Court has considered the pleadings filed in support of and in opposition to the motion and the remainder of the file and hereby denies the motion for the reasons stated herein.

I. PROCEDURAL HISTORY

On November 24, 2010, Plaintiff Syntrix Biosystems, Inc. ("Syntrix") filed a complaint against Illumina asserting numerous causes of action, including alleged infringement of United States Patent No. 6,951,682 (the "'682 Patent"). Dkt. 1.

On June 11, 2012, the Court issued a claim construction order. Dkt. 75. In that order, the Court declined to adopt Illumina's proposed limitations for the terms "gelled network," "particles," and "porous coating." With regard to the term "gelled network,"

the Court declined to construe the term to exclude "a planar, two-dimensional configuration of particles." *Id.* at 11–14. With regard to the term "particles," the Court declined to construe the term to exclude objects having a size greater than 1000 Å. *Id.* at 14–15.

On December 19, 2012, Illumina moved for summary judgment arguing in part that (1) the asserted claims were invalid and (2) the '682 Patent had a priority date no earlier than December 1, 1998. Dkt. 101 at 14–22. On February 7, 2013, the Court denied the motion on both of these issues because questions of fact existed for the jury. Dkt. 236 at 8–9.

In February and March of 2013, the Court held an eleven-day trial. On March 14, 2013, the jury returned a verdict in favor of Syntrix. Dkt. 285. Specifically, the jury found that the patent was infringed, the patent was not invalid, and that Syntrix had proved that it was entitled to a reasonable royalty of 6% for past infringement. *Id.*

II. FACTUAL BACKGROUND

The Court finds that an explanation of the facts of this case is unnecessary because the evidence challenged by Illumina represents a small portion of the trial. Therefore, the Court will cite relevant evidence throughout the discussion of the issues presented.

III. DISCUSSION

As a threshold matter, the Court will not review its claim construction rulings. Illumina contends that the Court should ensure that the verdict is supported by a "legitimate" reading of the jury charge (Dkt. 363 at 7) and the Court has the authority to clarify its rulings in the charge to the jury (Dkt. 370 at 10). These arguments are

essentially requests to read limitations into the asserted claims that the Court declined to adopt when it construed the claims. The claim construction order stands on its own, and the Court declines to do now what it declined to do then, which is add certain limitations to the asserted claims of the patent. Therefore, putting these arguments aside, the Court will address the sufficiency of the evidence.

Trial in this matter was mostly a battle of the experts. Both parties called highly qualified and highly accomplished individuals with extensive knowledge in the field of DNA microarrays. On the questions of fact before the Court, the jury sided with Syntrix's expert, Dr. Michael Metzker ("Dr. Metzker"). The verdict is the result of seven citizens' hard work and, as explained more fully below, is supported by the evidence. With regard to the claim constructions, the Court listened intently to each expert's position on how one of ordinary skill in the art would interpret the '682 Patent. While expert testimony is unlikely to be reliable in guiding the court's claim construction (*see Phillips v. AWH Corp.*, 415 F.3d 1303, 1319 (Fed. Cir. 2005)), Dr. Metzker's testimony was persuasive evidence that the Court's rulings were correct. Therefore, these issues will go to the Federal Circuit undisturbed because the Court is not persuaded that any errors exist in its construction of the claims.

A. JMOL

If "the court finds that a reasonable jury would not have a legally sufficient evidentiary basis to find for" a party on a particular issue, "the court may . . . resolve the issue against the party; and grant a motion for judgment as a matter of law . . . ." Fed. R. Civ. P. 50(a)(1). A motion for judgment as a matter of law "is not a patent-law-specific

issue, so regional circuit law applies." *Harris Corp. v. Ericsson Inc.*, 417 F.3d 1241, 1248 (Fed. Cir. 2005). In the Ninth Circuit, the district court must "view the evidence in the light most favorable to the nonmoving party . . . and draw all reasonable inferences in that party's favor." *Ostad v. Oregon Health Sciences University*, 327 F.3d 876, 881 (9th Cir. 2003). "Judgment as a matter of law is proper when the evidence permits only one reasonable conclusion and the conclusion is contrary to that reached by the jury." *Id.* (citing *Monroe v. City of Phoenix*, 248 F.3d 851, 861 (9th Cir. 2001).

1. "gelled network"

Illumina contends that "BeadChip does not meet the 'gelled network' requirement because it lacks the required 'aggregation of particles linked together to form a porous three-dimensional network.'" Dkt. 363 at 7 (citing Transcript ("Tr.") at 1779:4–6). Illumina separates its argument into two parts: (1) BeadChips do not contain a three-dimensional network and (2) BeadChips do not contain an aggregation of particles linked together. Dkt. 363 at 7–22. However, Syntrix's expert, Dr. Metzker, testified at length about how the Beadchips met both of these requirements. Tr. at 619:15–622:6. Although Dr. Metzker was subject to cross-examination and Illumina's expert, Dr. Milan Mrksich ("Dr. Mrksich"), provided contrary interpretations of the '682 Patent, Syntrix's evidence is sufficient to sustain the jury verdict. At the very least, the evidence supports more than one reasonable conclusion, one of which is that reached by the jury. Therefore, the Court denies Illumina's motion on this issue.

With regard to the supplemental briefing, the Court finds Illumina's position unpersuasive. While this motion was pending, the Federal Circuit issued its opinion in

Case 3:10-cv-05870-BHS Document 376 Filed 11/04/13 Page 5 of 10

SkinMedica, Inc. v. Histogen, Inc., 727 F.3d 1187 (Fed. Cir. 2013), in which it considered the term "culturing cells in three-dimensions." The court found that "the patentees plainly and repeatedly distinguished culturing with beads from culturing in three-dimensions." *Id.* at 1196. The court also found that the patentees "avoided anticipatory prior art during prosecution by asserting that the conditioned medium produced by two-dimensional cultures was inferior and chemically distinct from the conditioned medium produced by three-dimensional cultures." *Id.* As applied to this case, the Court finds that *SkinMedica* is factually distinguishable, specific to that patent in question, and does not provide a binding or persuasive rule such that the Court should alter its claim construction of the '682 Patent to exclude a monolayer of particles.

2. "porous coating"

Illumina challenges the evidence on this limitation on four issues: (1) the BeadChip does not have two distinct layers; (2) the BeadChip does not have a continuous coating; (3) the BeadChip does not have a substantially uniform thickness; and (4) the BeadChip does not have void regions. Dkt. 363 at 24–26. First, Dr. Metzker testified that the BeadChip had a silicon dioxide layer on top of a silicon layer, which is sufficient evidence to support the reasonable conclusion that the BeadChip contains a substrate and a porous coating. Tr. 547–549, 574. Therefore, the Court denies Illumina's motion on this issue.

Second, Illumina argues that the BeadChip does not have a "continuous" coating with "virtually no discontinuities or gaps." Dkt. 363 at 24–25. Dr. Metzker testified that a person of ordinary skill in the art would interpret this limitation to mean a coating with

90% material and 10% discontinuities or gaps. Tr. 597–598. The Court finds that 90% is not an unreasonable interpretation of "virtually." Moreover, the evidence that 99% of BeadChip products meet this interpretation of the limitation is sufficient evidence to support the verdict of infringement. With regard to the issue of Dr. Metzker changing his opinion from 98% during his deposition to 90% at trial, the Court finds that the alteration was adequately explained by Syntrix and does not undermine Dr. Metzker's credibility on this issue such that the evidence should be ignored as not credible. Therefore, the Court denies Illumina's motion on this issue.

Third, Illumina argues that the BeadChip does not have a substantially uniform thickness and that the reasonable interpretation of this limitation should be the height of the largest bead in the network compared to the height of an empty well, essentially 100% variation. Dkt. 363 at 25–26. This interpretation is either unreasonable, or, at the very least, one of many reasonable interpretations. On the other hand, Dr. Metzker's interpretation of a weighted average over the entire surface area of the coating was reasonable and supported by sufficient evidence that the BeadChip products met this limitation. Therefore, the Court denies Illumina's motion on this issue.

Fourth, Illumina argues that the BeadChip does not contain void regions in the porous coating. Dkt. 363 at 26–27. It is undisputed that there are void regions between the wells and the beads. What is disputed is whether the silicon dioxide layer containing the wells can be considered the porous coating. *See* Dkt. 367 at 27–28. This issue has already been resolved in Syntrix's favor. Therefore, the Court denies Illumina's motion on this issue.

3. Invalidity

Illumina argues that the '682 Patent is invalid for three reasons: (1) it lacks an adequate written description, (2) the priority date of the patent is no earlier than December 1, 1998, and (3) the disclosed subject matter is anticipated. Dkt. 363 at 27–36.

a. Adequate Written Description

The written description must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, the inventor was in possession of the invention, and demonstrate that by the disclosure in the specification. *Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351–52 (Fed. Cir. 2010) (en banc).

In this case, Illumina argues that the patent lacks an adequate written description because (1) the inventor disclaimed particles less than 1000 Å and (2) the patent does not describe monolayers of beads. Dkt. 363 at 27–30. These are both issues of law that were addressed in the Court's claim construction order. *See* Dkt. 75 at 11–15. The Court declines to reconsider that order. Therefore, the Court denies Illumina's motion on these issues.

b. Priority Date

Although Illumina's argument is titled that the priority date of the patent is "no earlier" than December 1, 1998, the substance of the argument is that the patent is not entitled to a priority date before the asserted prior art references, the Walt patents. *See* Dkt. 363 at 30–32. To enjoy a priority date earlier than a prior art reference, an inventor must prove conception before the priority date of that reference and reasonable diligence over the entire period from just before the priority date of the reference through the date

of his reduction to practice. *Griffith v. Kanamaru*, 816 F.2d 624, 626 (Fed. Cir. 1987). "[A]n inventor's testimony concerning his diligence [must] be corroborated." *Brown v. Barbacid*, 436 F.3d 1376, 1380 (Fed. Cir. 2006).

In this case, Illumina argues that Syntrix failed to produce sufficient evidence of corroboration. Dkt. 363 at 31. The Court disagrees. Dr. Zebala drafted the lengthy and complicated patent application. To accomplish this, he relied on a guide to drafting patents that showed signs of extensive use. Moreover, he testified that he relied on numerous other sources, some found on microfiche, which is corroborated by similar phrases in the '628 Patent. This evidence is sufficient to corroborate Dr. Zebala's diligence from at least the prior art priority date of September 11, 1998. It should also be noted that the prior art was reduced to practice in "late 1996" and, with the help of a patent attorney to draft the application, the application was filed in March of 1997. Tr. 1166–1169. This is credible and sufficient persuasive circumstantial evidence supporting Dr. Zebala's declared diligence because, in this complicated area of technology, an unskilled patent drafter would require at least as much time as a skilled patent drafter to draft an adequate application. Therefore, the Court denies Illumina's motion on this issue.

c. Anticipation

Illumina argues that the'628 Patent was anticipated by the Walt Patents. Dkt. 363 at 32–36. However, because the '628 Patent is entitled to a priority date before the Walt Patents, the Walt Patents are not prior art and may not be relied upon to invalidate the

'628 Patent as anticipated. *See* Dkt. 226 at 15. Therefore, the Court denies Illumina's motion on this issue.

4. Reasonable Royalty

Upon a showing of infringement, a patentee is entitled to "damages adequate to compensate for the infringement, but in no event less than a reasonable royalty for the use made of the invention by the infringer." 35 U.S.C. § 284. A "reasonable royalty" derives from a hypothetical negotiation between the patentee and the infringer when the infringement began. *ResQNet.com, Inc. v. Lansa, Inc.*, 594 F.3d 860, 868 (Fed. Cir. 2010). The burden of proving damages falls on the patentee. *Dow Chem. Co. v. Mee Indus., Inc.*, 341 F.3d 1370, 1381 (Fed. Cir. 2003). The patentee can meet that burden by producing evidence relating to the "comprehensive (but unprioritized and often overlapping) list of relevant factors for a reasonable royalty calculation [set forth] in *Georgia–Pacific Corp. v. United States Plywood Corp.,* 318 F. Supp. 1116, 1120 (S.D.N.Y. 1970)." *ResQNet.com*, 594 F.3d at 869.

In this case, Syntrix has met its burden of producing sufficient evidence to support the reasonable royalty of 6%. It is undisputed that this royalty rate was based off of Illumina's license with Tufts University. Pursuant to that agreement, Illumina licensed two Walt Patents, in addition to other patents. Illumina has consistently argued that the '682 Patent is anticipated and/or obvious in light of the Walt Patents, which essentially is an admission that the technologies are related. Having established the similarities of the technologies in question, the remaining issue is the difference between the 6% royalty the jury awarded and the 3% royalty in the Tufts License. On this issue, Syntrix's expert

explained the difference between a commercial license versus a license with a university, different bargaining positions when the technology is commercialized versus the situation where the licensee does not have a product on the market yet, and the separate agreements Dr. Walt negotiated with Illumina. Tr. 849–875. The Court finds that this is sufficient evidence to support a royalty rate higher than 3%, and the 6% royalty rate is not unreasonable. Therefore, the Court denies Illumina's motion on this issue.

B. New Trial

In the alternative to judgment as a matter of law, Illumina moves for a new trial. Dkt. 363 at 38–39. Illumina argues that the jury verdict goes against the great weight of evidence and that the jury instructions were erroneous insofar as they did not reflect the proper claim construction. *Id.* The Court disagrees because Syntrix produced sufficient evidence to support the verdict and the jury instructions were consistent with the Court's claim construction order. Therefore, the Court denies Illumina's motion for a new trial.

IV. ORDER

Therefore, it is hereby **ORDERED** that Illumina's motion for judgment as a matter of law or new trial (Dkt. 363) is **DENIED**.

Dated this 4th day of November, 2013.

BENJAMIN H. SETTLE
United States District Judge

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC.,

Plaintiff,

v.

ILLUMINA, INC.,

Defendant.

CASE NO. C10-5870 BHS

ORDER DENYING PLAINTIFF'S MOTION TO STRIKE AND GRANTING IN PART AND DENYING IN PART THE PARTIES' *DAUBERT* MOTIONS

This matter comes before the Court on Plaintiff Syntrix Biosystems, Inc.'s ("Syntrix") motion to strike (Dkt. 145) and motion to exclude (Dkt. 150); and Illumina, Inc.'s ("Illumina") motions to exclude (Dkts. 168 & 172). The Court has considered the pleadings filed in support of and in opposition to the motions and the remainder of the file and hereby denies the motion to strike and grants in part and denies in part the motions to exclude for the reasons stated herein.

I. PROCEDURAL HISTORY

On January 22, 2013, Syntrix filed a motion to exclude certain testimony of Dr. Milan Mrksich (Dkt. 150) and a motion to strike the supplemental information of Dr.

Mrksich (Dkt. 145); and Illumina filed a motion to exclude the opinions of Alan Ratliff (Dkt. 168) and Dr. Michael Metzker (Dkt. 172). On February 4, 2013, the parties responded. Dkts. 187, 192, 204, & 207. On February 8, 2013, Syntrix replied to Illumina's response to Syntrix's motion to strike. Dkt. 237.

II. DISCUSSION

A. Motion to Strike

Fed. R. Civ. P. 37(c)(1) "forbid[s] the use at trial of any information required to be disclosed by [Fed. R. Civ. P.] 26(a) that is not properly disclosed." *Hoffman v. Constr. Protective Servs., Inc.*, 541 F.3d 1175, 1179 (9th Cir. 2008) (quoting *Yeti by Molly, Ltd. v. Deckers Outdoor Corp.*, 259 F.3d 1101, 1106 (9th Cir. 2001)). The party facing sanctions bears the burden of proving that its failure to disclose the required information was substantially justified or is harmless. *Torres v. City of L.A.*, 548 F.3d 1197, 1213 (9th Cir. 2008).

In this case, Syntrix argues that the supplemental report of Dr. Mrksich should be stricken and that he should not be allowed to testify about its contents. Dkt. 145 at 2. Illumina responds that the information provided does not alter Dr. Mrksich's substantive opinions, the information was timely produced, and, even if there is a violation of the rules, the error is harmless and can be corrected by additional depositions before trial. Dkt. 207. The Court agrees that any possible violation can be mitigated. During the pretrial conference, pursuant to the Court's suggestion, the parties agreed to cure the alleged violation of Dr. Metzker's supplemental information via a subsequent deposition and cross-examination. It's arguable that Dr. Metzker provided a wholly new opinion as

infringement whereas Dr. Mrksich has only provided new raw numbers relating to the infringing products, which may be entered into previously disclosed calculations. Solving problems associated with new opinions is generally more difficult than solving problems associated with new data. Therefore, the Court denies Syntrix's motion to strike and exclude Dr. Mrksich's supplemental disclosure because the Court finds that the parties may resolve any problem with subsequent depositions and cross-examination.

B. *Daubert* Motions

Expert testimony is admissible if it is "scientific, technical, or other specialized knowledge" that "will help the trier of fact to understand the evidence or to determine a fact in issue," and "the testimony is based on sufficient facts or data . . . the testimony is the product of reliable principles and methods; and the expert has reliably applied the principles and methods to the facts of the case." Fed. R. Evid. 702. The court acts as a gatekeeper to ensure that expert testimony "is both relevant and reliable." *Avila v. Willits Envtl. Remediation Trust*, 633 F.3d 828, 836 (9th Cir. 2011). The court may apply four nonexclusive factors to determine whether proffered expert opinion is developed by the scientific method or is "junk science":

> District court judges are to consider not only (1) whether the method has gained general acceptance in the relevant scientific community, but also (2) whether the method has been peer-reviewed, (3) whether the method "can be (and has been) tested," and (4) whether there is a "known or potential rate of error." [*Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 594 (1993)] . . . . [T]he Daubert inquiry is flexible . . . . "One very significant fact" is whether the expert has "developed [his] opinions expressly for purposes of testifying," since "a scientist's normal workplace is the lab or the field, not the courtroom or the lawyer's office." [*Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 43 F.3d 1311, 1317 (9th Cir.), *cert. denied*, 516 U.S. 869 (1995) ("*Daubert II*")]. That the expert failed to

> subject his method to peer-review and to develop his opinion outside the litigation is not dispositive, but if these guarantees of reliability are not satisfied, the expert "must explain precisely how [he] went about reaching [his] conclusions and point to some objective source . . . to show that [he has] followed the scientific method, as it is practiced by (at least) a recognized minority of scientists in [his] field." *Id.*

Lust v. Merrell Dow Pharmaceuticals, Inc., 89 F.3d 594, 597 (9th Cir. 1996) (quoting *Daubert II*, 43 F.3d at 1317–1319. "[T]he test under Daubert is not the correctness of the expert's conclusions but the soundness of his methodology." *Daubert II*, 43 F.3d at 1318. The gatekeeping function applies to all expert opinions, whether based on specialized, technical, or scientific knowledge. *Kumho Tire Co.., Ltd. v. Carmichael*, 526 U.S. 137, 148–49 (1999). We "determine reliability in light of the particular facts and circumstances of the particular case." *Id.* at 158.

1. Dr. Mrksich

Syntrix moves to exclude certain testimony of Dr. Mrksich for numerous reasons. Dkt. 150. To the extent that Syntrix moves to exclude based on the information provided in Dr. Mrksich's supplement produced January 16, 2013, the Court denies the motion because it is repetitive of Syntrix's motion to strike. The Court will more substantively address the remaining issues.

First, Syntrix argues that Dr. Mrksich "skews the data in such a manner that makes it unreliable and not representative of the actual BeadChips." Dkt. 150 at 6. Illumina counters that "Syntrix argument makes no sense." Dkt. 204 at 3. The Court agrees to the extent that Syntrix has failed to show that data is skewed to an unreliable, irrelevant extent.

Second, Syntrix argues that Dr. Mrksich's model includes unrealistic end points that would allow for the physical impossibility of the beads lying below the bottom of the well. Dkt. 150 at 7–9. Illumina, however, has cited the portion of Dr. Mrksich's report that accounts for negative position heights. Dkt. 204 at 6. Therefore, this appears to be a nonissue.

Third, Syntrix argues that Dr. Mrksich's testimony should be limited to the actual alleged infringing products that he tested, which was not all of the products. Dkt. 150 at 9. Illumina concedes that Dr. Mrksich will testify "consistent with the disclosures he made in his report." Dkt. 204 at 7. Therefore, this appears to be a nonissue.

Fourth, Syntrix argues that Dr. Mrksich presents unreliable information regarding 2-micron BeadChips. Dkt. 150 at 10. Illumina "contends that the cited documents adequately support Dr. Mrksich's opinion on the issue of 'process agents.'" Dkt. 204 at 8. Illumina, however, is incorrect. The first document does not support the conclusion of losses over 20% because it shows losses of only 19.7%. The second document is a red-line revision of comments to a manufacturing process explaining improvement to the 19.7% loss. The Court finds that Syntrix has met its burden to exclude paragraphs 160 & 161 from Dr. Mrksich's report as well as any testimony on these opinions.

Fifth, Syntrix argues that Dr. Mrksich presents no evidence regarding Accused Infringing Assays. Dkt. 150 at 11. Illumina contends that Dr. Mrksich may present evidence that this product is not infringed because the infringement of this product requires infringement of an independent claim and Dr. Mrksich has analyzed products under the independent claim. Dkt. 204 at 9. Moreover, Illumina contends that Dr.

Mrksich has provided opinion on Illumina's assays that are not directly linked to Syntrix's patent. *Id.* Therefore, the Court denies Syntrix's motion on this issue.

Finally, Syntrix moves to exclude Dr. Mrksich's report and testimony as to Illumina's argument of non-infringement on the "continuous" limitation and affirmative defenses of anticipation and obviousness. Dkt. 150 at 12–14. These issues have been squarely addressed in the Court's order denying the parties' summary judgment motions. *See* Dkts. 183 at 5–8 (affirmative defenses) & 236 at 7. Based on the reasoning contained therein, the Court denies Syntrix's motion.

Therefore, the Court grants in part and denies in part Syntrix's motion to exclude the report and testimony of Dr. Mrksich.

2. Dr. Metzker

Illumina moves to exclude Dr. Metzker's opinions on infringement, information relating to Syntrix's state law claims, and the enablement of certain pieces of prior art. Dkt. 172.

a. "Substantially Uniform Thickness"

Illumina argues that the Court should exclude Dr. Metzker's opinion on the "substantially uniform thickness" limitation because it "is not based on sufficient facts or data and is not the product of reliable principles and methods." Dkt. 172 at 5. The Court construed this limitation in part to require that "the thickness of the coating varies by no more than 30% over the entire coated area." Dkt. 75 at 6. It's undisputed that the protrusion height of particles above the substrate is one of the determinative factors as to whether the accused products fall within the scope of this limitation. Syntrix contends

that "[u]nsuprisingly, the experts disagree on which statistical data should be used to calculate the protrusion height." Dkt. 192 at 3. Syntrix goes on to show that Illumina's problems with Dr. Metzker's opinion go to the weight and are not the type of fundamental problems that should result in exclusion of the evidence altogether. *Id*. at 3–6. The Court agrees and, therefore, denies Illumina's motion on this issue.

b. Failure to Follow Claim Construction Order

The Court has discretion to exclude expert testimony "as irrelevant because it [is] based on an impermissible claim construction . . . ." *Liquid Dynamics Corp. v. Vaughn Co., Inc.*, 449 F.3d 1209, 1224 n.2 (Fed. Cir. 2006).

In this case, Illumina argues that portions of Dr. Metzker's opinion should be excluded as irrelevant because they are based on an improper claim construction. Dkt. 172 at 5–12. With regard to the "gelled network" limitation and the linking of the particles, Illumina's arguments were denied on summary judgment. Dkt. 236 at 5.

With regard to the "substrate" limitation, Illumina's arguments are confusing. First, Illumina appears to argue that Dr. Metzker's opinions are irrelevant because he treats optional embodiments as requirements of the claim. *See, e.g.*, Dkt. 172 at 8 ("Dr. Metzker treats flatness as a requirement."). The Court declines to exclude these opinions because whether specific embodiments or products characteristic of specific embodiments are encompassed within a claim limitation is relevant evidence. On the other hand, if Dr. Metzker attempts to narrow the claim limitation to specific embodiments to overcome indefiniteness or prior art (Dkt. 172 at 8), then the testimony

may be irrelevant and inadmissible. This possible issue, however, may be adequately handled during trial.

Second, Illumina argues that "Dr. Metzker's analysis of the 'substrate' limitation is also unreliable because it ignores obviously relevant portions of the patent's 'Substrate Selection and Preparation' discussion." Dkt. 172 at 9. Illumina contends that it is not a "competent" analysis and that it "is not good enough to support an expert opinion." *Id.* at 9–10. These issues, however, are subjects of cross-examination because they go to the weight of the evidence and not the admissibility. Therefore, the Court denies the motion on the "substrate issue."

With regard to the "known discrete full thickness volume" limitation, Illumina basically argues that Dr. Metzker may not offer testimony that narrows the limitation to overcome indefiniteness. Dkt. 172 at 10–12. Although the Court agrees, this does not make his opinions as to how the accused product falls within the scope of the claims irrelevant or unreliable. Therefore, the Court denies Illumina's motion on this issue.

c. Information Relating to State Law Claims

Illumina moves to exclude a number of "improper opinions relating to" Syntrix's state law claims. Dkt. 172 at 12. Syntrix argues that the opinions that support the now dismissed state law claims also support Syntrix's position that the patent is not invalid and claim for willful infringement. Dkt. 192 at 12–15. The Court agrees to a certain extent. For example, Dr. Metzker's opinions as to secondary considerations of non-obviousness are admissible and will help the jury to understand the issue. Dkt. 192 at 13. Moreover, Dr. Metzker's opinions as to the extent of technical information that was

disclosed to Illumina and the similarities between the disclosed information and the Dickson application are relevant to willful infringement and will help the jury understand the issue of willfulness. *Id.* at 14. On the other hand, the jury does not need an expert opinion on issues such as the similarities between the words "Syntrix" and "Sentrix." Dkt. 172 at 15–16. Therefore, the Court denies the motion to exclude all of this evidence with the understanding that specific objections will be addressed when inadmissible testimony is proffered.

d. Enablement

For a reference to anticipate: "[f]irst, the reference must disclose each and every element of the claimed invention, whether it does so explicitly or inherently." *In re Gleave*, 560 F.3d 1331, 1334 (Fed. Cir. 2009). "Second, the reference must enable one of ordinary skill in the art to make the invention without undue experimentation." *Id.* (quoting *Impax Labs., Inc. v. Aventis Pharms. Inc.*, 545 F.3d 1312, 1314 (Fed. Cir. 2008)) (internal quotation marks omitted).

In this case, Illumina argues that Dr. Metzker "applied the incorrect standard in reaching his opinions that the prior art references were not enabling." Dkt. 172 at 18. The Court agrees as to some opinions. For example, Dr. Metzker opines that "Chee II does not enable one of ordinary skill in the art . . . [because] there were no actual reductions to practice of any of the embodiments described in the application." Ex. B ¶ 203. This opinion is improper because a valid patent does not require an actual reduction to practice as it is widely known that an individual may possess an enforceable patent without possessing an actual product. In other words, the law only requires sufficient

disclosure to enable one to reduce the invention to practice. *See In re Gleave*, 560 F.3d at 1334.

Syntrix, however, contends that when read in the context of the entire section of Dr. Metzker's opinion, the contested opinion is admissible testimony. Dkt. 192 at 17. The Court disagrees as no context will save the incorrect statement. Therefore, the Court grants the motion on this opinion and any other opinion of prior art based on this particular reasoning. The Court notes that Dr. Metzker offers other admissible opinions on the issue of anticipation, and the Court is confident that the attorneys will seek to offer only those opinions at trial.

3. Mr. Ratliff

Illumina moves to exclude the report of Mr. Ratliff, Syntrix's damages expert. Dkt. 168. Illumina contends that (1) Mr. Ratliff relies on licenses that have no demonstrative link to the technology at issue; (2) Mr. Ratliff improperly supports his rate determination; and (3) Mr. Ratliff improperly speculates as to Illumina's license with Tufts University. *Id.* at 5. Syntrix counters that Illumina's "complaints 'go to the weight of the testimony and not its admissibility.'" *Activision Networks, Inc. v. Verizon Comm'ns, Inc. et al.*, 694 F.3d 1312, 1333 (Fed. Cir. 2012). The Court agrees.

First, Mr. Ratliff relied on previous expert testimony that the licenses in question were related to DNA microarrays. If Illumina elects to attack that link or the prior expert testimony at trial, then it may. But this is not a sufficient reason to exclude Mr. Ratliff's report and testimony.

Second, Illumina contends that Mr. Ratliff improperly considers Dr. David Walt's personal stake in Illumina. Dkt. 168 at 9–12. However, the inference that Tufts University received a lower royalty rate from Illumina in return for its doctor receiving a partial stake in Illumina is reasonable. Attacking this inference goes to the weight of Mr. Ratliff's opinion and not to any fundamental deficiency.

Finally, Illumina contends that Mr. Ratliff should have considered the price of Illumina stock when Tufts bought the stock ($5,000) and not the value when Tufts sold the stock ($7.7 million). Dkt. 168 at 12–13. The inference that Tufts agreed to a lower royalty rate based on a gamble that Illumina would be successful is a reasonable inference as is the fact that Illumina was actually successful. Attacking this inference and fact goes to the weight and not to any fundamental deficiency. Therefore, the Court denies Illumina's motion to exclude Mr. Ratliff's testimony and opinion.

III. ORDER

Therefore, it is hereby **ORDERED** that Syntrix's motion to strike (Dkt. 145) is **DENIED** and the parties' motions to exclude (Dkts. 150, 168, & 172) are **GRANTED in part** and **DENIED in part** as stated herein.

Dated this 15th day of February, 2013.

BENJAMIN H. SETTLE
United States District Judge

UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WASHINGTON
AT TACOMA

SYNTRIX BIOSYSTEMS, INC.,

Plaintiff,

v.

ILLUMINA, INC.,

Defendant.

CASE NO. C10-5870 BHS

ORDER GRANTING PLAINTIFF'S MOTION FOR JUDGMENT AND MOTION FOR ONGOING ROYALTIES

This matter comes before the Court on Plaintiff Syntrix Biosystems, Inc.'s ("Syntrix") motion for judgment (Dkt. 313) and motion for ongoing royalties (Dkt. 330). The Court has considered the pleadings filed in support of and in opposition to the motions and the remainder of the file and hereby grants the motions for the reasons stated herein.

I. PROCEDURAL HISTORY

On November 24, 2010, Syntrix filed a complaint against Defendant Illumina, Inc. ("Illumina") asserting numerous causes of action, including alleged infringement of United States Patent No. 6,951,682. Dkt. 1. On March 14, 2013, the jury returned a

verdict in favor of Syntrix, which included a finding that Syntrix had proved that it was entitled to a reasonable royalty of 6% for past infringement. Dkt. 285.

On May 1, 2013, Syntrix filed a motion for judgment. Dkt. 313. On May 10, 2013, Illumina responded. Dkt. 334.

On May 3, 2013, Syntrix filed a motion for ongoing royalties. Dkt. 330. On May 31, 2013, Illumina responded. Dkt. 344. On June 10, 2013, Syntrix replied. Dkt. 348.

II. DISCUSSION

A. Judgment

Both parties agree that a judgment should issue including the verdict, the matters of supplemental damages, prejudgment interest, and ongoing royalties. Illumina disputes, however, whether taxation of costs should be included in the judgment. Dkt. 334 at 2. The Court agrees with Illumina and taxation of costs should be requested post judgment. Therefore, the Court grants Syntrix's motion with the exception of costs.

B. Ongoing Royalties

Following a jury verdict of infringement, a district court may award an ongoing royalty for continued patent infringement. *See Paice LLC v. Toyota Motor Corp.*, 504 F.3d 1293, 1315 (Fed. Cir. 2007). The parties should ordinarily be given an opportunity to negotiate a license regarding future use of the patented invention, but if they are unable to reach agreement, the district court can "step in to assess a reasonable royalty in light of the ongoing infringement." *Id.*

An ongoing royalty is a form of equitable relief authorized under 35 U.S.C. § 283. *See id.* at 1315 n. 16. The Court is not bound by the prejudgment royalty rate found by

the jury. *Amado v. Microsoft Corp.*, 517 F.3d 1353, 1361–62 (Fed. Cir. 2008). "[P]re-suit and post-judgment acts of infringement are distinct, and may warrant different royalty rates given the change in the parties' legal relationship and other factors." *Paice*, 504 F.3d at 1317 (Rader, J., concurring).

In *Read Corp. v. Portec, Inc.*, 970 F.2d 816 (Fed. Cir. 1992), the Federal Circuit provided guiding factors to determine whether and how much damages should be enhanced in light of Defendant's ongoing willful infringement. These factors include: (1) whether the infringer deliberately copied the ideas or design of another; (2) whether the infringer, when he knew of the other's patent protection, investigated the scope of the patent and formed a good-faith belief that it was invalid or that it was not infringed; (3) the infringer's behavior as a party to the litigation; (4) the infringer's size and financial condition; (5) the closeness of the case; (6) the duration of the infringer's misconduct; (7) any remedial action taken by the infringer; (8) the infringer's motivation for harm; and (9) whether the infringer attempted to conceal its misconduct. *See id.*, 970 F.2d at 827.

In this case, Syntrix seeks an ongoing royalty instead of a permanent injunction. Syntrix, however, requests that the Court impose a 9% royalty rate for ongoing infringement instead of the 6% rate the jury found for past infringement. Dkt. 330. While the parties dispute the evidence and how it influences the *Read* factors, Syntrix has failed to show that any particular factor or combination of factors weighs in favor of a higher royalty rate for ongoing infringement. The one persuasive argument that Syntrix makes is the parties' relative bargaining positions post-verdict. Although the Court has continually expressed that there are close questions of law in this case, the jury quickly

and soundly rejected Illumina's positions. After an eleven-day trial, the jury returned a complete verdict for Syntirx in two and an half hours. *See* Dkt. 284. Based on this decisiveness and the evidence offered at trial, the Court finds that Syntrix is in a stronger bargaining position than it was prior to the verdict. Additionally, Illumina has no readily apparent alternative if it seeks to continue its production and sale of the infringing product. The Court further finds that a 2% increase in the ongoing royalty rate adequately compensates Syntrix for the stronger bargaining position. Therefore, the Court grants Syntrix's motion and imposes an 8% royalty rate for ongoing infringement.

The Court notes that Illumina contends that its status as an infringer should not change post verdict because close questions of law are still in play in this proceeding. The Court agrees as to the existence of the questions of law, but disagrees as to Illumina's status. If Illumina is successful with its post judgment motions, then the ongoing royalty rate is irrelevant. If Illumina is unsuccessful, then it is undisputed that Illumina has been fully heard on all of its positions and both the judge and jury have rejected those non-infringement and invalidity positions. Such finality undercuts Illumina's positions, and Syntrix is placed in a stronger position to negotiate the rate for additional infringing sales. Therefore, the Court declines to adopt Illumina's argument that the parties' status has not changed despite the near finality of this proceeding.

Finally, the parties also dispute how the rate should be calculated. Syntrix requests that the rate be imposed on orders while Illumina contends that the royalties should be based on shipments. The Court agrees with Illumina. Therefore, ongoing royalties shall be calculated based on products shipped.

III. ORDER

Therefore, it is hereby **ORDERED** that Syntrix's motion for judgment (Dkt. 313) is **GRANTED** and motion for ongoing royalties (Dkt. 330) is **GRANTED**. Syntrix shall submit a proposed order for ongoing royalties consistent with this order.

Dated this 18th day of June, 2013.

BENJAMIN H. SETTLE
United States District Judge

US006951682B1

(12) **United States Patent**
Zebala

(10) **Patent No.: US 6,951,682 B1**
(45) **Date of Patent: Oct. 4, 2005**

(54) **POROUS COATINGS BEARING LIGAND ARRAYS AND USE THEREOF**

(75) Inventor: **John A. Zebala**, Redmond, WA (US)

(73) Assignee: **Syntrix Biochip, Inc.**, Auburn, WA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/332,815**

(22) Filed: **Sep. 17, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/110,529, filed on Dec. 1, 1998.

(51) **Int. Cl.**[7] **B32B 3/10**; B32B 3/26; C12Q 1/00; G01N 33/53; G01N 33/543

(52) **U.S. Cl.** **428/312.2**; 428/321.6; 428/312.8; 428/315.7; 428/317.1; 428/195; 435/4; 435/7.1; 435/7.7; 435/7.71; 435/7.72; 435/7.8; 435/7.9; 436/518; 436/519; 436/523

(58) **Field of Search** 428/195, 312.2, 428/312.6, 312.8, 315.5, 315.7, 317.1, 317.9, 318.4, 319.1; 435/4, 7.1, 7.7, 7.71, 7.72, 7.8, 7.9, 7.91, 7.92, 7.93, 7.94, 7.95; 436/518, 519, 523

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,757,014 A * 7/1988 Hendrickson et al. 435/180
5,001,453 A * 3/1991 Ikejiri et al. 338/35
6,159,681 A * 12/2000 Zebala 435/4

FOREIGN PATENT DOCUMENTS

EP WO 98/41534 * 9/1998

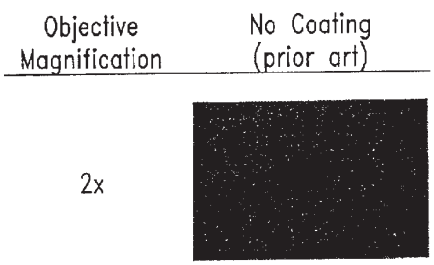
* cited by examiner

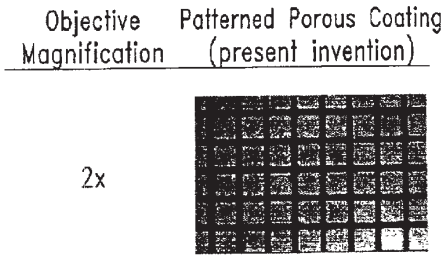
Primary Examiner—Cheryl A. Juska
(74) *Attorney, Agent, or Firm*—Peter J. Knudsen

(57) **ABSTRACT**

Articles comprising substantially uniform porous coatings, which may be photopatterned, are provided. The use of such porous coatings increases the surface density of attached compounds within, for example, ligand arrays prepared by methods such as regionally selective solid-phase chemical synthesis. Arrays prepared using the porous coatings may be used within a variety of diagnostic and drug discovery assays.

155 Claims, 9 Drawing Sheets





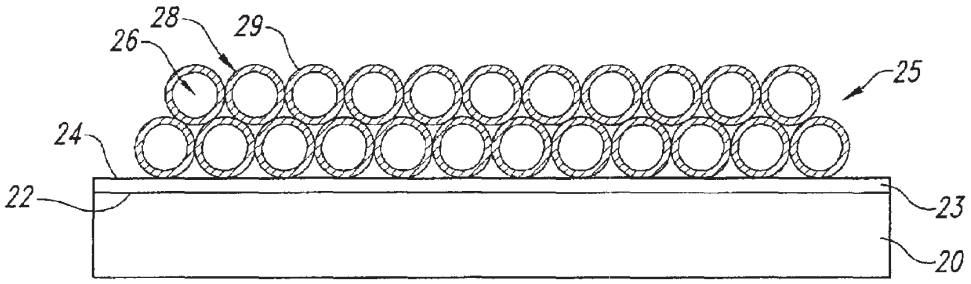


Fig. 1A

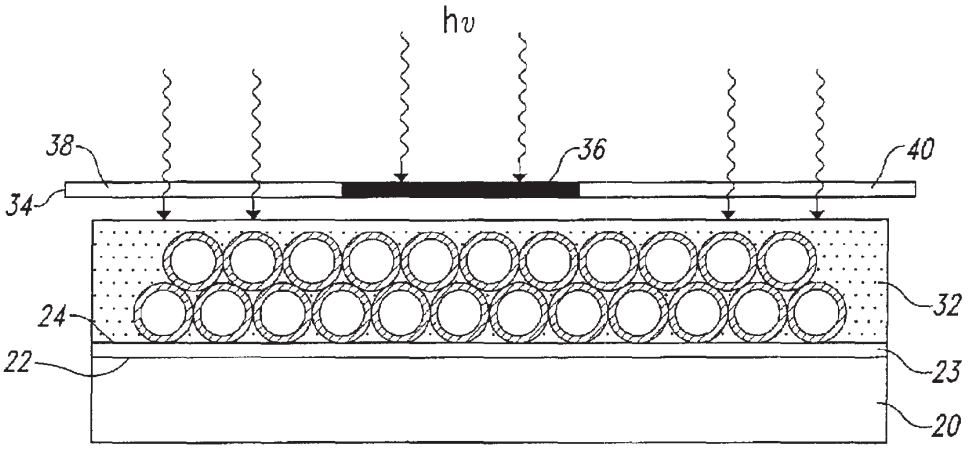


Fig. 1B

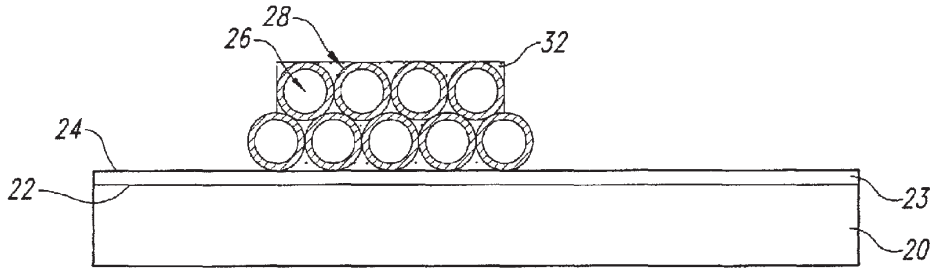


Fig. 1C

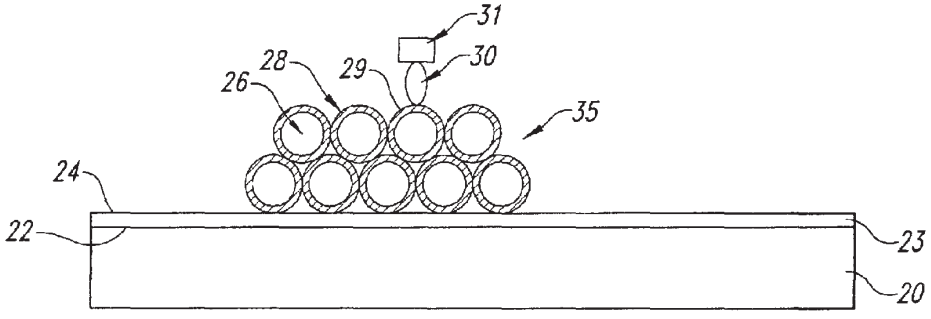


Fig. 1D

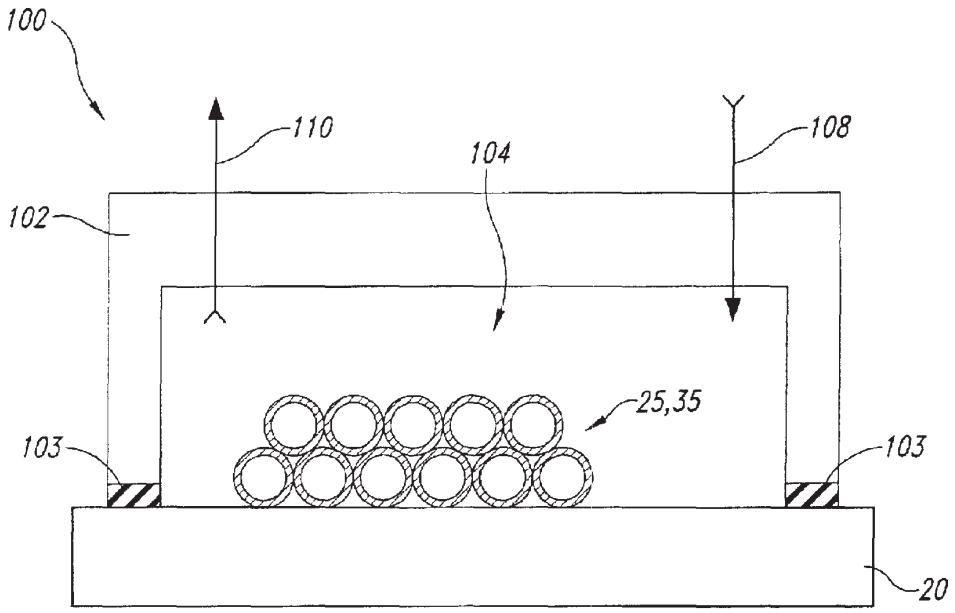


Fig. 1E

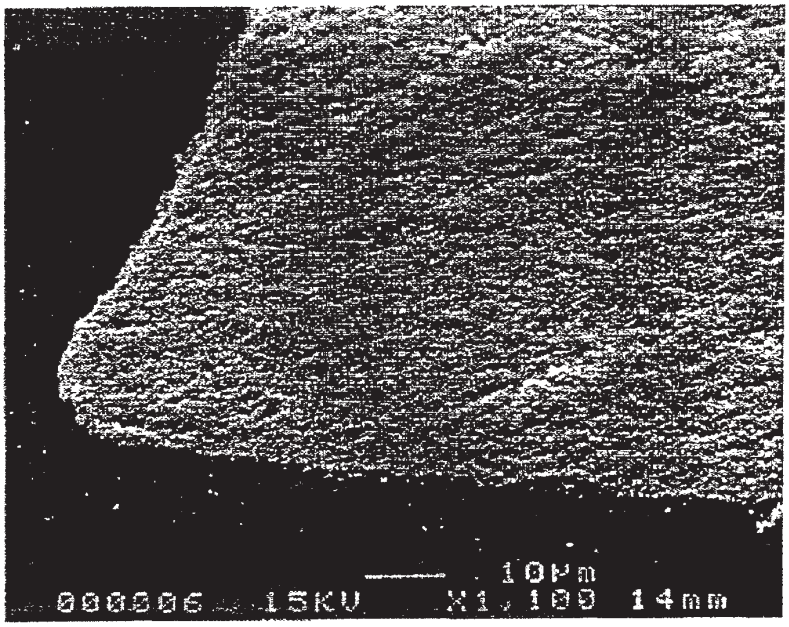


Fig. 2

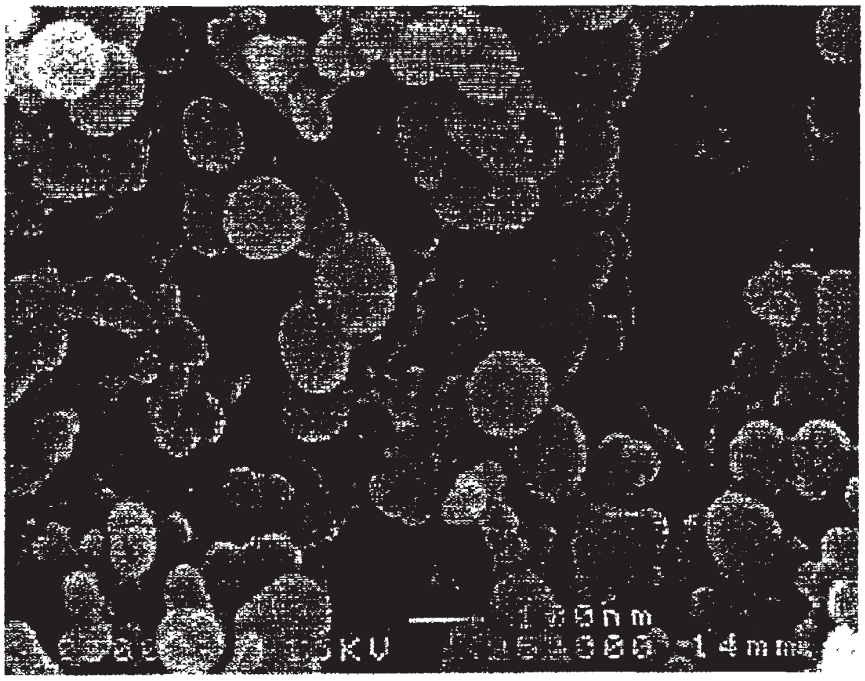


Fig. 3

Objective Magnification	No Coating (prior art)
2x	

Fig. 4A

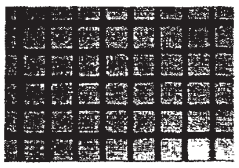
Objective Magnification	Patterned Porous Coating (present invention)
2x	

Fig. 4B

Objective Magnification	No Coating (prior art)
10x	

Fig. 4C

Objective Magnification	Patterned Porous Coating (present invention)
10x	

Fig. 4D

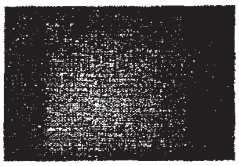
Objective Magnification	No Coating (prior art)
20x	

Fig. 4E

Objective Magnification	Patterned Porous Coating (present invention)
20x	

Fig. 4F

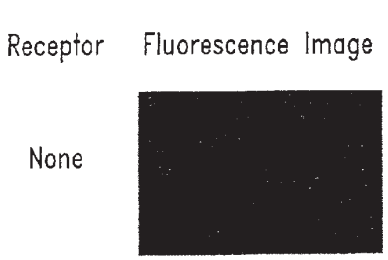


Fig. 5A

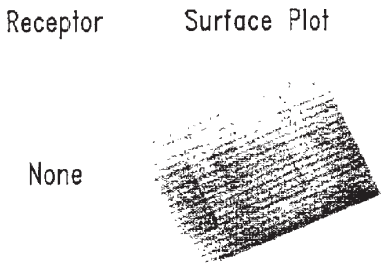


Fig. 5B

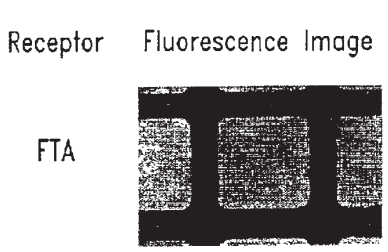


Fig. 5C

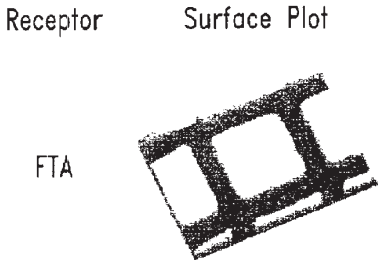


Fig. 5D

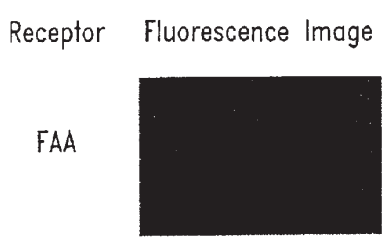


Fig. 5E

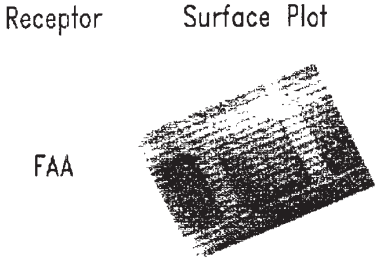


Fig. 5F

PNA array contacted by FAA receptor

Receptor Sequence 5' – GCGAAGGC - F

Array schematic

AT	GT	CT	TT
AC	GC	CC	TC
AG	GG	CG	TG
AA	GA	CA	TA

Fig. 6A

PNA array contacted by FTA receptor

Receptor Sequence 5' – GCGTAGGC – F

Array schematic

AT	GT	CT	TT
AC	GC	CC	TC
AG	GG	CG	TG
AA	GA	CA	TA

Fig. 6B

PNA array contacted by FAA receptor

Fluorescence Image

Fig. 6C

PNA array contacted by FTA receptor

Fluorescence Image

Fig. 6D

PNA array contacted by FAA receptor

Surface Plot

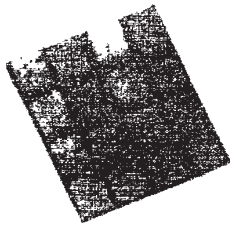


Fig. 6E

PNA array contacted by FTA receptor

Surface Plot

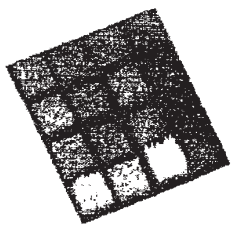
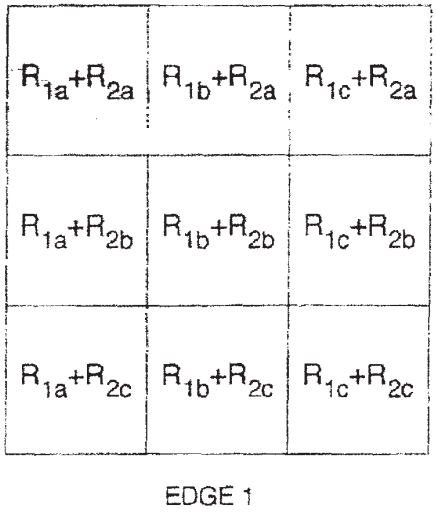


Fig. 6F

Array Schematic



Surface Plot

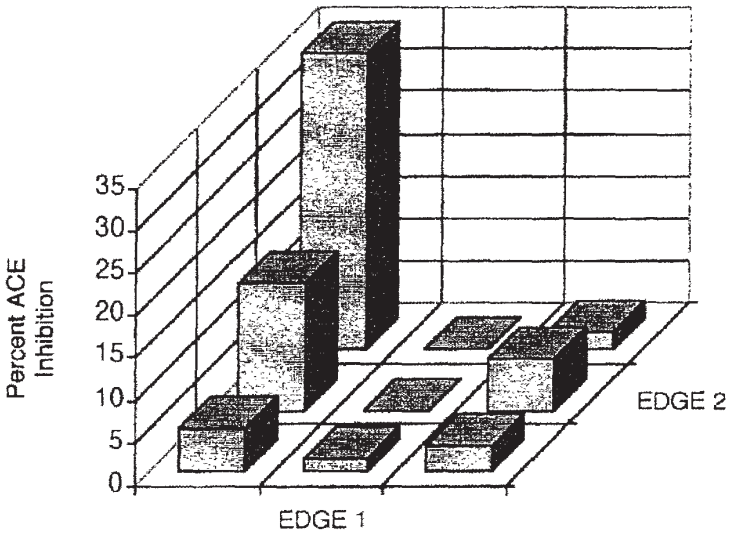


Fig. 7

POROUS COATINGS BEARING LIGAND ARRAYS AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This Application claims the benefit of U.S. Provisional Application No. 60/110,529, filed Dec. 1, 1998.

TECHNICAL FIELD

The present invention relates generally to articles comprising porous coatings, and to methods for preparing and using such articles. The invention is more particularly related to methods for fabricating articles having patterned porous coatings, which may be used, for example, to screen large numbers of discrete compounds for diagnostic or drug discovery purposes.

BACKGROUND OF THE INVENTION

Receptor-ligand interactions are critical components of many fundamental biological processes. Such interactions involve specific binding of a macromolecule receptor (e.g., enzyme, cell-surface protein, antibody or oligonucleotide) to a particular ligand molecule. Receptor-ligand binding may affect any of a variety of intercellular and intracellular processes in an organism, such as signal transduction, gene expression, immune responses or cell adhesion. An improved understanding of receptor-ligand interactions is necessary for many areas of research in the life sciences, as well as for the development of agents that modulate such interactions for therapeutic and other applications.

Miniaturized ligand-arrays, formed using microfabrication and solid-phase chemical synthesis on substantially planar supports, have been used to facilitate the study of receptor-ligand interactions (for representative examples, see Fodor et al., Science (1991) 251:767; Pease et al., *Proc. Natl. Acad. Sci. USA* 91:5022, 1994; Pirrung et al., U.S. Pat. No. 5,405,783; Fodor et al., U.S. Pat. No. 5,445,934; Pirrung et al., U.S. Pat. No. 5,143,854; Fodor et al., U.S. Pat. No. 5,424,186 and Fodor et al., U.S. Pat. No. 5,510,270; Chee et al., Science (1996) 274:610 and Brennan, U.S. Pat. No. 5,474,796). Contacting a ligand array with labeled receptor allows many ligands to be simultaneously screened for receptor binding. The location of bound receptor on the array is determined by detecting photons or radioactivity. However, the surface density of ligand is often low, resulting in the need for costly imaging equipment and long image acquisition times. Drug discovery efforts have been further hampered by low ligand surface density, since many functional assays require higher ligand concentrations to identify drug leads.

One approach to increasing surface density of ligands involves immobilizing ligands on an array of polyacrylamide pads using microfabrication techniques (see Guschin et al., *Anal. Biochem.* 250:203, 1997 and Yershov et al., *Proc. Natl. Acad. Sci. USA* 93:4913, 1996). Such an approach increases the surface density of the ligands, but places a size restriction on diffusion into the polymer that many receptors exceed. Furthermore, such polymeric supports may not be compatible with solid-phase chemical synthesis, which requires adequate swelling and salvation of a polymeric matrix in order to achieve efficient mass transfer of reagents. Further, although this polymer can be photopatterned (i.e., multiple discrete pads may bc generated by a process involving exposure to irradiation), the photosensitivity is severely limited, requiring 30 minutes of illumination. Such a low throughput is inadequate for mass production.

Existing techniques for increasing ligand density on a solid support provide insufficient surface area enhancement. Such techniques include the use of acid-etched porous silicon and an electrochemically manufactured metal oxide membrane as substrates for detecting the specific binding of ligands by receptors (see Beattie et al., *Clin. Chem.* 41:700, 1995 and Van Damme and Kreuwel, WO99/02266). The porous silicon is macroporous with 3 to 5 micron diameter pores arranged in parallel and oriented perpendicular to the substrate surface. Relative to nanoporous materials, a macroporous configuration has inadequate surface area to significantly increase ligand surface density. Although the electrochemically manufactured metal oxide membrane has pores as small as 0.2 microns, it too provides little surface area enhancement with only a 10-fold increase in surface area for each micron of membrane thickness.

Additionally, the parallel pore orientation of these substrates is technically cumbersome, since it requires a flow-through apparatus in order for receptor to bind ligand. Further, it is unclear whether such substrates could function as solid supports for multiple rounds of synthetic reactions. The electrochemically manufactured metal oxide membrane also suffers from incompatibility with microfabrication methods.

Rigid porous supports that do not require swelling in solvents and are compatible with attachment of ligands or receptors offer the potential to increase ligand surface density by providing a high surface area for ligand attachment. For example, porous bodies have been made from slurries consisting of a binder and particles having a high surface area (see Messing, U.S. Pat. No. 3,910,851 and Messing, in: Methods in *Enzymology*, vol. XLIV, p. 149, edited by Klaus Mosbach, (1976), Academic Press N.Y.). However, to date, porous supports and coatings have not been successfully applied to microfabrication of ligand arrays.

Porous coatings with controlled porosity have been obtained by sol-gel and particulate methods (see Frye et al., U.S. Pat. No. 5,224,972; Frye et al., U.S. Pat. No. 5,589,396; Suppiah, U.S. Pat. No. 5,120,600 and Frye et al., in: *Better Ceramics Through Chemistry IV*, vol. 180, Mat. Res. Soc. Symp. Proc., edited by Brinker et al., (1990), p. 583). Such methods produce controlled porous coatings with chemically modified surfaces for the purpose of providing steric and chemical selectivity to a sensor surface, via nonspecific molecular interactions (e.g. chelation and ion exchange). Such coatings have not been used as supports for detecting the specific binding characteristic of macromolecular receptors or to create arrays of complex ligands. Further such coatings cannot be made greater than one micron thick without multiple coats, and have not been successfully patterned by microfabrication methods.

Other porous coatings suffer from incompatibility with solid phase ligand synthesis. From the field of imaging, positive and negative images can be formed in coatings of photosensitized colloidal particles (see Pu et al., *J Imaging Sci.* 33:177, 1989). Such coatings consist of a phenolic resin (0% to 15%), a bis-azide (optional), and colloidal particles encapsulated by organic polymer, diacid chlorides, and photoactive azide groups. Although these coatings may be patterned using microfabrication techniques, they have not been used to increase ligand surface density, detect ligand-receptor binding or prepare ligand arrays by solid-phase chemical synthesis. In fact, organic solvents would be expected to swell and distort existing coatings, making them incompatible with solid-phase synthesis.

Still further porous inorganic coatings have been designed to reduce reflectivity. For example, both aged and unaged

colloidal dispersions have been used to form continuous porous coatings of uniform thickness (see Cathro et al., *Solar Energy* 32:573, 1984 and Lange et al., U.S. Pat. No. 4,816,333). The resulting dried coatings are from about 0.02 μm to 0.50 μm thick. Although the surface area of a porous coating may be increased by increasing its thickness, uniform colloidal coatings greater than about 1.5 μm thick cannot be obtained without using certain additives (see Daniels et al., in: *Better Ceramics Through Chemistry VII*, vol. 435, Mat. Res. Soc. Symp. Proc., edited by Coltrain et al., (1996), p. 215). Even with additives, such coatings still form cracks. In fact, colloidal coatings typically are non-uniform and discontinuous (see Moulton, U.S. Pat. No. 2,601,123). Further, colloidal coatings have not been patterned using microfabrication techniques, or used to increase ligand surface density, detect ligand-receptor binding, or prepare ligand arrays by solid-phase chemical synthesis.

Accordingly, there is a need in the art for methods for increasing ligand density on a surface in a manner that is fully compatible with microfabrication. In particular, there is a need for improved articles for use in the detection of macromolecular receptor binding, and the production of ligand arrays by solid-phase synthetic methods. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides articles comprising porous coatings and attached ligands, and methods for preparing and using such articles. Within certain aspects, the present invention provides coated articles comprising a substrate having a continuous porous coating of substantially uniform thickness, wherein the coating comprises a gelled network of particles, and wherein the porous coating has two or more different compounds attached thereto. Suitable particles may comprise one or more of carbon, activated carbon, fluorinated carbon, styrenedivinylbenzene copolymers, polystyrene, zeolites, oxides of antimony and oxides of metals present within Group III and Group IV of the Periodic Table. The primary particle size is preferably less than 1000 Å. Within certain embodiments, the gelled network of particles further comprises a polymer of a partially or substantially hydrolyzed metal alkoxide. Substrates include glass and may, but need not, comprise an adhesive layer. Attachment of compounds to the porous coating may be covalent or via adsorption, with or without the use of a linker. Preferred compounds include nucleobase polymers, peptides and enalaprilat analogues.

Within further aspects, methods are provided for making a coated article with two or more compounds attached thereto, comprising the steps of: (a) applying to a substrate a substantially uniform layer of a solution comprising metal oxide particles dispersed in a volatile liquid; (b) evaporating the volatile liquid from the layer, forming a gelled network of metal oxide particles on the substrate, wherein the gelled network forms a porous coating ranging from 0.05 to 25 microns thick; and (c) attaching two or more compounds to discrete known regions of the porous coating. The solution may further comprise extended polymers of a substantially hydrolyzed metal alkoxide linked to the metal oxide particles, wherein the weight ratio of metal oxide particles to the substantially hydrolyzed metal alkoxide ranges from 1 to 1000. Optionally, prior to the step of attaching two or more compounds, the porous coating is cured at a temperature and for a time sufficient to increase the porous coating strength.

Within further aspects, the present invention provides coated articles comprising a substrate having at least two discrete known regions with porous coatings, wherein each coating has a substantially uniform thickness, is continuous and comprises a gelled network of particles, and wherein each porous coating has at least one compound attached thereto. Suitable particles may comprise one or more of carbon, activated carbon, fluorinated carbon, styrenedivinylbenzene copolymers, polystyrene, zeolites, oxides of antimony and oxides of metals present within Group III and Group IV of the Periodic Table. The primary particle size is preferably less than 1000 Å. Within certain embodiments, the gelled network of particles further comprises a polymer of a partially or substantially hydrolyzed metal alkoxide. Substrates include glass and may, but need not, comprise an adhesive layer. Attachment of compounds to the porous coating may be covalent or via adsorption, with or without the use of a linker. Preferred compounds include nucleobase polymers, peptides and enalaprilat analogues.

The present invention further provides methods for making a coated article comprising a substrate and at least two separate porous coatings, comprising the steps of: (a) applying to a substrate a substantially uniform layer of a solution comprising metal oxide particles dispersed in a volatile liquid; (b) evaporating the volatile liquid from the layer, forming a gelled network of metal oxide particles on the substrate, wherein the gelled network forms a porous coating ranging from 0.05 to 25 microns thick; (c) covering the porous coating with a layer of photoresist comprising a base soluble component; (d) irradiating the photoresist, such that a first region of photoresist is rendered substantially removable with an aqueous alkaline developer, and such that a second region is not so removable; (e) contacting at least the first region with an aqueous alkaline developer to remove at least the first region of photoresist and porous coating underlying the first region, without substantially removing the second region of photoresist or porous coating underlying the second region; (f) removing remaining photoresist with an organic solvent, resulting in separate porous coatings on discrete regions of the substrate; and (g) attaching one or more compounds to each of the separate porous coatings. The solution may further comprise extended polymers of a substantially hydrolyzed metal alkoxide linked to the metal oxide particles, wherein the weight ratio of metal oxide particles to the substantially hydrolyzed metal alkoxide ranges from 1 to 1000. Optionally, prior to the step of attaching two or more compounds, the porous coating is cured at a temperature and for a time sufficient to increase the porous coating strength.

Within further aspects, the present invention provides methods for identifying at least one compound that specifically binds a receptor, the method comprising the sequential steps of: (a) contacting a coated article as described above with a receptor; and (b) determining whether one or more of the compounds attached to the porous coating specifically bind to the receptor.

The present invention further provides methods for identifying at least one compound that specifically binds a receptor, the method comprising the steps of: (a) simultaneously or in either order (i) detaching one or more compounds from a coated article as described above; and (ii) contacting the detached compound(s) with a receptor; and (b) determining whether the compound(s) specifically bind to the receptor.

Within other aspects, methods are provided for isolating a target receptor, comprising the steps of: (a) contacting a coated article as described above with a composition comprising a target receptor, wherein at least one attached compound binds to the target receptor; (b) removing

unbound components of the composition from the array; and (c) separating the target receptor from the coated article.

Methods are further provided for modifying a receptor, comprising contacting a coated article as described above with a composition comprising a target receptor, wherein at least 5% of the attached compounds comprise a target receptor modifying group that labels, reconforms, cleaves, covalently binds or intercalates into a bound target receptor.

Within other aspects, the present invention provides methods for hybridizing an antisense molecule to a target nucleic acid molecule, comprising the steps of: (a) contacting a coated article as described above with a composition comprising a target nucleic acid molecule, wherein the attached compounds are antisense molecules; and (b) detaching one or more compounds from the array.

Methods are further provided, within other aspects, for hybridizing an antisense molecule to a target nucleic acid molecule, comprising the steps of: (a) detaching one or more compounds from a coated article as described above, wherein the attached compounds are antisense molecules; and (b) contacting the compound(s) with a composition comprising a target nucleic acid molecule.

The present invention further provides coated articles comprising a substrate having a continuous porous coating thereon of substantially uniform thickness, wherein the porous coating comprises a continuous gelled network of metal oxide particles and polymers of hydrolyzed metal alkoxide, wherein the porosity of the coating ranges from 0.15 to 0.99.

Within other aspects, the present invention provides coated articles comprising a substrate having at least five separate distinct porous coatings per square centimeter, wherein each coating is continuous and has a substantially uniform thickness and comprises a continuous gelled network of particles.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A is a diagram illustrating a cross-section of a representative article comprising a porous coating **25** with an attached ligand **31** and linker **30**. Surface **22** of substrate **20** is provided with an adhesive layer **23** having an adhesive surface **24**. The porous coating **25** is substantially uniform in thickness, and comprises a continuous gelled-network of substantially spherical metal oxide particles **26** and a metal alkoxide polymer **28**. The surface area is formed by the porous coating surface **29**.

FIG. **1**B is a diagram illustrating a cross-section of a representative article as shown in FIG. **1**A, following application of photoresist layer **32** and during irradiation. An opaque region **36** of mask **34** is used to block light radiation to a first region of photoresist layer **32**. Transparent regions **38** and **40** of the mask allow light to irradiate second and third regions of the photoresist layer, respectively. The thickness of photoresist layer **32** is sufficient to substantially cover porous coating **25** and fill its pore volume.

FIG. **1**C is a diagram illustrating a cross-section of a representative article as shown in FIG. **1**B, following removal of irradiated photoresist and underlying porous coating with a developer. Remaining photoresist **32** substantially covers the remaining gelled-network of substantially spherical metal oxide particles **26** and a metal alkoxide polymer **28**.

FIG. **1**D is a diagram illustrating a cross-section of a representative article as shown in FIG. **1**C after stripping the photoresist and attaching a ligand **31** via a linker **30**.

FIG. **1**E is a diagram illustrating a cross-section of a reactor system **100** for applying liquid reagents to a surface of a porous coating. Reactor system **100** is formed by mating substrate **20** to a reactor base **102** with an intervening gasket **103**. Sandwiched together, the substrate, gasket, and reactor base form a sealed reactor cavity **104** except for an inlet port **108** and an outlet port **110**. The reactor cavity is in contact with porous coating **25** or a patterned porous coating **35**.

FIG. **2** is a scanning electron microscope print at a magnification of 1,100× that shows a portion of a patterned porous coating according to the present invention. The scale-bar at the bottom of the print is 10 μm long.

FIG. **3** is a scanning electron microscope print of the porous coating in FIG. **2** at a magnification of 95,000×. Minute granularities on the surface of the metal oxide particles are due to sputtered metal deposited on the specimen as part of the preparation process for viewing with the scanning electron microscope. The scale-bar at the bottom of the print is 100 nm long.

FIGS. **4**A–**4**F are prints from an epifluorescence microscope at objective magnifications of 2×(FIGS. **4**A and **4**B), 10×(FIGS. **4**C and **4**D), and 20×(FIGS. **4**E and **4**F) that show ligand surface density according to an existing method (i.e., no coating; FIGS. **4**A, **4**C and **4**E), and using a representative porous coating as provided herein (FIGS. **4**B, **4**D and **4**F). The ligand is FITC attached to the free amino group of aminopropyltriethoxysilane bound to the surface.

FIGS. **5**A–**5**F are prints (FIGS. **5**A, **5**C and **5**E) and surface plots (FIGS. **5**B, **5**D and **5**F) from an epifluorescence microscope that demonstrate specific binding of ligand by a fluorescently labeled receptor on a representative patterned porous coating, wherein both the ligand and receptor are DNA. The ligand was synthesized on the patterned porous coating by solid-phase synthetic methods. The receptor used was: no receptor (FIGS. **5**A and **5**B), FTA (FIGS. **5**C and **5**D) or FAA (FIGS. **5**E and **5**F).

FIGS. **6**A–**6**F are schematics (FIGS. **6**A and **6**B), epifluorescence microscope prints (FIGS. **6**C and **6**D) and surface plots (FIGS. **6**E and **6**F) illustrating the specific binding of a ligand array by two different fluorescently labeled receptors (FAA or FTA, as indicated) on a patterned porous coating according to the present invention, wherein both receptors are DNA, and the ligand array is a peptide nucleic acid (PNA) array. The symbol "F" indicates fluorescein. Shaded grids on the ligand array schematics indicate the predicted location of receptor binding. The ligand array was synthesized on the patterned porous coating by photolithography and solid-phase synthetic methods.

FIGS. **7**A and **7**B are a schematic (FIG. **7**A) and a plot of enzyme inhibition (FIG. **7**B) from an array of weakly inhibitory ligands synthesized on a representative patterned porous coating, wherein the enzyme is angiotensin converting enzyme (ACE), and the ligands are analogues of enalaprilat, the active metabolite of the antihypertensive drug enalapril. The ligand array was synthesized on the patterned porous coating by photolithography and solid-phase synthetic methods. The surface plot illustrates percent ACE inhibition as a function of array position.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to articles comprising porous coatings. The present

invention is based, in part, on the discovery that a uniform, crack-free porous coating up to 25 μm thick can be generated in which pore size may be tailored so as to optimize both surface area and mass transfer characteristics. Such coatings increase ligand surface density with low autofluorescence, and can be applied and patterned with high sensitivity using microfabrication techniques. Porous coatings provided herein are compatible with the production of ligand arrays by solid-phase synthesis, do not swell or distort substantially during ligand-receptor binding or solid-phase chemical synthesis and do not require a flow-through apparatus, resulting in economical and rapid imaging of ligand arrays. The enhanced ligand surface density provided by the coatings described herein is sufficient to perform functional assays using ligands from individual array elements, and with low to moderate binding affinities.

Porous coatings provided herein may be used, for example, to prepare arrays of ligands (e.g., nucleobase polymers). Ligand arrays as described herein may be used in analyses that require a large number of discrete compounds on a solid support, such as within screens to detect ligand-receptor binding for diagnostic or drug discovery purposes.

Glossary

Prior to setting forth the invention in detail, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

An "acid labile moiety" is a portion of a molecule that is cleaved upon exposure to a particular acidic chemical or pH. Similarly, a "base labile moiety" is a portion of a molecule that is cleaved upon exposure to a particular basic chemical or pH.

An "adhesive layer" is a coating that is stably attached to the surface of a substrate to facilitate adhesion of a porous coating to the substrate. Stable attachment may be assessed by a modification of ASTM Test Method D3330, wherein a 1.9 cm wide strip of Scotch Brand Magic transparent tape, available from 3M company, is adhered to the test layer by rolling a 2 kg roller back and forth twice across the tape. The tape is then peeled from the test sample at 1800 at a rate of 2 cm/min. Stable attachment is defined as a 180° peelback value of greater than about 25 g/cm, and more preferably greater than about 150 g/cm. The ability of an adhesive layer to facilitate porous coating attachment may be readily assessed by the modified ASTM Test Method D3330 as described above. An adhesive layer is said to facilitate porous coating attachment if it has a 180° peelback value of greater than about 15 g/cm, or if there is a split in the porous coating leaving residual porous coating on the adhesive layer. In certain preferred embodiments, the adhesive layer comprises partially, substantially or fully hydrolyzed monomers, oligomers, and/or extended polymers of a metal oxide or organo-metal alkoxide. Within such polymers, coupling is achieved through "oxane" bonds (i.e., "-M-O-M-"; condensation products of: -M-OH+OH-M-→M-O-M-+H_2O, where M is a metal) In another preferred embodiment, the adhesive layer comprises a substantially hydrolyzed tetraethyoxysilane. In some embodiments the thickness of the adhesive layer is less than 0.001 μm, 0.01 μm, 0.1 μm, 1.0 μm, or 10.0 μm. Preferably, the thickness is between 0.1 μm and 1.0 μm.

"Aging" of a composition refers to the process of forming extended polymers according to the sol-gel method. Such polymers may be linear or crosslinked polymers. Aging may proceed in the liquid (i.e., "sol"), gel and/or solid states, and generally refers to the period over which the number of condensed chemical bonds is increasing. Bond condensation may reach an equilibrium in the liquid, gel or solid states. Bond condensation or "aging" may be monitored using techniques described below (see "extended polymer"). A sol is said to be "aged" when bond condensation has progressed to yield extended polymers. Aged solutions described in this specification are preferably aged until an equilibrium of bond condensation is reached in the liquid state. Equilibrium may be detected using techniques described below (see "extended polymer").

"Amplification" refers to a detectable increase in the number of copies of a particular nucleic acid fragment or other biologic molecule, usually resulting from an enzymatic reaction such as the polymerase chain reaction (PCR).

An "antisense molecule" is a nucleobase polymer that has a sequence that is at least partially complementary to a nucleic acid molecule of interest, and which detectably modulates the expression and/or activity of the nucleic acid via hydrogen bonding interactions. Also encompassed are nucleobase polymers that are candidates for possessing such modulating activity (e.g., an array of antisense molecules may comprise multiple nucleobase polymers that are to be screened for antisense properties). The ability to modulate nucleic acid activity by antisense regulation is well known in the art (reviewed in Uhlmann and Peyman, *Chem. Rev.* 90(4):544, 1990 and Schreier, *Pharm. Acta Helv.* 68(3):145, 1994). With respect to the control of gene expression, antisense molecules can be used not only to inhibit expression, but also to activate it in vitro and in vivo. Indirect activation of gene expression can be accomplished, for example, by suppressing the biosynthesis of a natural repressor, as described for antisense oligodeoxynucleotides by Inoue (see Inoue, *Gene* 72:25, 1988) Direct activation of gene expression can be accomplished, for example, by reducing termination of transcription as described for antisense oligodeoxynucleotides by Winkler et al. (see Winkler et al., *Proc. Natl. Acad. Sci. USA* 79:2181, 1982). There are several in vitro and in vivo test systems known in the art that have been routinely used (see Crooke, *Anticancer Drug Des.* 6:609, 1991; Hanvey et al., *Science* 258:1481, 1992; Liszewicz et al., *Proc. Natl. Acad. Sci. USA* 89.11209, 1992; Woolf et al., *Proc. Natl. Acad. Sci. USA* 89:7305, 1992; Nielsen et al., *Anticancer Drug Des.* 8:53, 1993 and Zelphati et al., *Antisense Res. Dev.* 3:323, 1993). The efficacy of antisense molecules in a ligand-array can be easily tested and compared using these test systems.

A compound is said to be "attached" to a substrate surface if the compound substantially remains on the surface during photoresist application and removal (i.e., at least 60% of the attached compounds are not removed when such processes are performed as described herein). The percentage of compounds removed under particular conditions may be readily determined using labeled molecules, and monitoring the loss of label during photoresist application and removal. Attachment may be covalent or non-covalent. Noncovalent interactions that may be employed include, for example, electrostatic interactions, hydrogen bonding, metal coordination, Van der Waals interactions, and magnetism. In some embodiments, a mixture of covalent and noncovalent interactions may be used. Suitable magnetizing agents for use in a magnetic field include paramagnetic lanthanide ions such as erbium, dysprosium, holmium, thulium, and gadolinium (see Zborowski et al., *J. Gen. Microbiology* 138:63, 1992; Russell et al., *Analytical Biochem.* 164:181, 1987; and Evans and Tew, *Science* 213:653, 1983). Alternatively, micron-scale and smaller magnetic affinity particles may be used such as ferritin, dextran magnetite, and magnetic porous glass (see Hirschbein et al., *Chemtech* pg. 172, March, 1982; Virooncharapan et al., *Pharm. Res.* 12:1176, 1995; and CPG Inc., Lincoln Park, New Jersey).

A "barrier layer" is a layer of photoresist that prevents detectable contact of a reagent on one side of the layer with a molecule on the other side over a time required for a particular reaction. In other words, a reagent that reacts in a detectable manner with a molecule when the two are combined in solution should not react detectably when separated from the molecule by a barrier layer. In some embodiments the barrier layer will be absolute, preventing detectable contact independent of time. Absolute barrier layers are preferably 0.1 to 20 microns thick, and more preferably 1 to 3 microns thick. In other embodiments the barrier layer will provide a relative diffusion barrier that prevents detectable contact over a specified time interval and specified barrier thickness. In the case of a relative diffusion barrier, a suitable barrier thickness will be determined empirically taking into account the required time of the reaction. In general, the barrier thickness and time interval are directly proportional to one another. That is, reactions requiring longer time intervals will require thicker barrier layers.

Two molecules are said to "bind" if they associate non-covalently such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art. A first molecule is said to "specifically bind" relative to a second unrelated molecule if the ratio of the first molecule's binding constant to the second molecule's binding constant is greater than 2, and preferably greater than 5.

The term "complementary" refers to electronic topologic compatibility or matching together of interacting surfaces of a ligand molecule and its receptor, resulting in detectable binding using an appropriate assay technique. Thus, a receptor and its ligand can be described as complementary, as can the contact surface characteristics of a receptor and its ligand. Depending on the degree of complementarity of two ligands for a particular receptor as exhibited by their binding constants, one ligand may be said to more specifically bind relative to the other (see "bind" above). Two nucleobase polymers are said to be "complementary" if the polymers are able to pair (as in Watson-Crick base-pairing) with corresponding bases in a given nucleic acid molecule of interest. The term "exactly complementary" indicates that 100% of the nucleobases in a particular sequence are able to engage in base-pairing with corresponding bases of a nucleic acid molecule of interest. The term "substantially complementary" indicates that at least about 80% of the nucleobases in a particular sequence are able to engage in base-pairing with corresponding bases of a nucleic acid molecule of interest. The term "partially complementary" indicates that at least about 60% of the bases in a particular sequence are able to engage in base pairing with corresponding bases of a nucleic acid molecule of interest.

A "compound" is any molecule including, but not limited to, ligands, receptors, nucleobase polymers and peptides.

A photoresist layer is "continuous" if virtually no straight-line penetrable discontinuities or gaps are detectable in the coating. In other words, such discontinuities or gaps should make up less than 30% of the layer, as detected using, for example, standard microscopy, phase-contrast microscopy, and fluorescence microscopy. It will be apparent that a layer need be continuous only over regions where such coating is necessary for preparation or use of a porous coating. Any number of discontinuities and gaps can exist in other regions.

A layer of photoresist is said to "cover" molecules attached to a surface if the layer forms a continuous coating that is at least 0.1 micron thick.

"Curing" refers to the process of gelation and densification that occurs after evaporation of a coating solution (see "sol-gel" below). During curing, the number of oxane bonds increases, which in turn increases the strength that metal oxide particles have between one another and the substrate. Curing may be accomplished by heating at high temperatures for short periods, or low temperatures for long periods. High temperatures are limited to those below the sintering temperature where individual particles melt and the porosity of a coating is reduced to zero. The degree of oxane bonding during any point of the curing process may be tested and monitored using differential thermal analysis, thermogravimetric analysis, and density measurements (see Villegas and Navarro, *J Material Sci.* 23:2142, 1988). In general, curing of a porous coating should be performed at a temperature and for time sufficient to detectably increase the strength (ie., the stable attachment) of the porous coating, using methods as described above, and preferably the coating strength is increased to a level desired for subsequent process steps.

Exposure of a photoresist to a "developer" may refer to any treatment that dissolves an irradiated portion of a positive photoresist or an unirradiated portion of a negative photoresist, permitting selective removal of the dissolved regions. A developer may be a liquid or gas composition. Certain preferred developers comprise a non-aqueous mixture of solvents containing various ratios of ketone, amino, hydroxyl and amide moieties. Alternatively, a developer may be irradiation. A photoresist is said to be exposed to developer if a developer composition is contacted with the photoresist, or if irradiation is targeted to the photoresist, such that the photoresist is substantially removed in a specific region.

A "discrete known region" is a localized area of a surface on which a substantially pure group of compounds is, was, or is intended to be attached. Such regions do not overlap. A discrete known region may have any convenient shape including circular, rectangular, elliptical, etc., and may be of any size, such as 0.25 to 10^6 square microns.

An "enzyme-cleavable moiety" is a portion of a molecule that is cleaved by exposure to a particular enzyme.

A solvent is said to be "evaporated" if less than 5% of the original solvent remains in the liquid state.

"Extended polymer" refers to the formation of a polymer by sol-gel (see "sol-gel" below). The progress of polymerization may be monitored by measuring, for example, the hydrodynamic radius by quasi-elastic light scattering, gas adsorption-desorption on sol-gel-coated surface acoustic wave (SAW) sensors, time dependent changes during NMR spectroscopy (e.g., ^{29}Si), and monitoring the H_2O-content of the reacting system using IR-spectroscopy [see Brinker et al., *Thin Solid Films* (1991) 201:97; Daniels et al., Mat. Res. Soc. Symp. Proc. (1996) 435:215 and Schmidt et al., J. Non-Cryst. Solids (1982) 48:65]. Other methods for monitoring the progress of polymerization will be apparent to those of skill in the art. An extended polymer according to the present invention will have an average hydrodynamic radius greater than 2 nm, more preferably greater than 8 nm, and most preferably greater than 16 nm.

A "fortifying solution" is a solution of a polymeric binder that may be applied to a porous coating during preparation to yield a "fortifying layer" on the surface of the porous

coating (see description of polymeric binder in "gelled network" below). The fortifying layer is typically applied before curing of the porous coating, and provides enhanced anchoring of the porous coating to the substrate. Enhanced anchoring may be assessed by an increase in the 180° peelback value using a modification of ASTM Test Method D3330 as described above for "adhesive layer " In a preferred embodiment, the fortifying layer comprises substantially hydrolyzed tetraethyoxysilane. The weight ratio of metal oxide particles to the fortifying layer plus other polymeric binder ranges from 1 to 1000.

"Full thickness volume" refers to the volume of a coating region as defined by the boundaries of the surface plane, the base plane (i.e., the plane of the coating in contact with the substrate surface or the surface of an adhesive layer), and the region. For example, a rectangular region of dimensions 1 and w on a coating of thickness t will have a full thickness volume of 1×w×t. A circular region of radius r will have a full thickness volume of $^{1}r^{2}t$ on the same coating.

"Gelled network" refers to an aggregation of particles linked together to form a porous three-dimensional network. Particles may be linked covalently or noncovalently through the use of a polymeric binder. Alternatively, particles may be linked covalently or noncovalently without the use of a binder, through interactions of chemical groups on the surface of the particles. Covalent interactions between polymeric binders or surface groups include the formation of, for example, oxane bonds (e.g., —O—Si—O—, —O-Ti-O—, —O—Al—O—, —O—B—O—, —O—Zr—O—, —O—Er—O—, —O—Cr—O——O—Ga—O—, —O—Ge—O—, O—Hf—O—, —O—Fe—O—, —O—Ca—O—, —O—Cr—O—, —O—La—O—, —O—Mg—O—, —O—Nb—O—, —O—K—O—, —O—Pr—O—, —O—Sm—O—, —Na—O—, —O—Ta—O—, —O—Te—O—, —O—Tl—O—, —O—Sn—O—, —O—W—O—, —O—V—O—, —O—Y—O—, and —O—Zn—O—), linkages between an epoxide (e.g., glycidoxypropyltrimethoxysilane) and a polyamine (e.g., triethylene tetramine), and photoinduced linkages using, for example, a bis-azide. Noncovalent interactions that may be employed in polymeric binders or surface groups include, for example, electrostatic interactions, hydrogen bonding, metal coordination, and Van der Waals interactions. In some embodiments, particles will be linked by a mixture of covalent and noncovalent interactions. The extent of linking sufficient to constitute a "gelled network" will be such that less than 20%, and more preferably less than 5%, of the network is lost after contact with any process agent (e.g., irradiation, photoresist, developers, strippers and reagents). Accordingly, the extent of linking required will depend on the exact nature of the process agents. For example, photoresists that exhibit higher degrees of swelling will require gelled networks with higher degrees of linking so as to balance the forces of swelling and prevent physical disruption of the gelled network. The percent loss of the network after contact with process agents can be readily assessed using nitrogen adsorption isotherms and the Brunauer-Emmett-Teller (BET) method. The BET method allows the surface area of the gelled network to be accurately measured, and the percent change in surface area after contact with a process agent will be equivalent to the percent loss of the gelled network. Other methods for assessing the percent loss of the gelled network after contact with process agents will be apparent to one of ordinary skill in the art.

"Hybridization" refers to the base-pairing or aggregation of one nucleobase polymer to another nucleobase polymer via complementary regions. The polymers may be, for example, DNA, PNA, morpholino-based nucleobase polymers and/or other nucleobase polymers. Such base-pairing or aggregation should be detectable using standard assays (e.g., detection of a marker linked to one nucleobase polymer). Whether or not a particular nucleobase polymer remains base-paired or aggregated with a target nucleobase polymer depends on the degree of complementarity, the length of the aggregated elements, and the stringency of the binding conditions. At a higher stringency, hybridization requires a higher degree of complementarity or length.

"Hydrolyzed" refers to the lysis of water to split a chemical bond. For example, a metal alkoxide may be partially hydrolyzed (i.e., from 10% to 50% of hydrolyzable bonds hydrolyzed) or substantially hydrolyzed (i.e., greater than 50% of hydrolyzable bonds hydrolyzed). For example, 1 equivalent of water will hydrolyze 2 equivalents of hydrolyzable bonds in tetraethoxysilane resulting in a metal alkoxide that is partially hydrolyzed. This is consequence of the 1 equivalent of water hydrolyzing 1 equivalent of hydrolyzable bonds that generate 1 equivalent of free silanols. The 1 equivalent of free silanols in turn condense and release water, which goes on to hydrolyze the remaining 1 equivalent of hydrolyzable bonds for a total of 2 equivalents of hydrolyzable bonds hydrolyzed.

"Irradiation" refers to the application of radiation to a target. The amount of irradiation depends on the desired result of the irradiation. In general, irradiation is sufficient to achieve a desired chemical modification on an irradiated molecule. For example, irradiation of a positive photoresist layer is sufficient to permit substantial removal of photoresist from irradiated regions.

A "label" or "marker" is a modification of a compound (e.g., a ligand or receptor) that enables the user to specifically detect the labeled compound in the presence of unlabeled compounds. For example, one or more atoms within the compound may be replaced with radioactive isotopes. Alternatively, labels may provide antigenic determinants, nucleic acids available for hybridization, altered fluorescence-polarization or altered light-scattering. Still other markers include those that are chromogenic, fluorescent, chemiluminescent or electrochemically detectable. Other methods available to label a ligand or receptor will be readily apparent to those skilled in the art.

A "ligand," as used in this specification, is any molecule that is a candidate for specific binding by a particular receptor. It will be understood that many ligands will not specifically bind their intended receptor. For example, the majority of ligands in a drug analogue array will not be expected to bind their target receptor specifically. Further, the term "ligand" is not limited to molecules having any particular biological function. Ligands may be considered to be members of the larger generic group termed "compounds," which also includes molecules that are not candidates for specific recognition by receptors. Ligands may be naturally-occurring or man-made molecules, and they can be employed in their unaltered state or as aggregates with other species. Ligands may be attached (covalently or non-covalently) to a surface, either directly or via other molecules, such as linkers and/or spacers. Ligands may covalently or non-covalently modify a given receptor after binding the receptor. Such modifications include labeling, altering conformation, cleaving, covalently binding and intercalation. A ligand that is capable of modifying a target receptor in such a manner is said to comprise a "target receptor modifying group." Examples of ligands include, but are not restricted to, agonists and antagonists for

cell membrane receptors, toxins and venoms, viral epitopes, hormones, antibodies, cell membrane receptors, monoclonal antibodies, antisera reactive to specific antigenic determinants, enzymes, drugs, drug analogues, polynucleotides, nucleic acid, catalytic nucleic acids, peptides, catalytic peptides, peptide nucleic acids, morpholino-based nucleobase polymers, other nucleobase polymers, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes and organelles.

A "ligand-array" is a two dimensional matrix of ligands attached to a surface.

"Ligand-receptor binding" refers to specific, detectable binding between a ligand and receptor through molecular recognition.

A "ligand-receptor pair" is a complex formed when a ligand and receptor bind through molecular recognition.

"Mask" refers to a substantially transparent support material with substantially opaque regions in a precise pattern where it is desired that light be blocked when one side of the mask is illuminated. In some embodiments the substantially opaque regions are derived through a photographic process using a photoplotting device (e.g., as in masks commonly used in printed circuit board manufacturing). In other embodiments the mask is derived from a substantially transparent support material coated with a substantially opaque material which is photoablated by a narrowly focused laser producing precisely defined transparent regions (e.g., chrome on glass masks). The differential between the intensity of light transmitted by substantially transparent and substantially opaque regions as a percentage of the intensity of light transmitted by substantially transparent regions should be greater than 75%, more preferably greater than 90%, and most preferably greater than 99%.

"Nucleic acid molecules" (or "nucleic acids") are polymers of nucleotides (i.e., compounds formed of phosphoric acid (H_3PO_4), a sugar, and a purine or pyrimidine base). Such polymers may be of any length, and include DNA and RNA molecules. Relatively short nucleic acid molecules (i.e., containing fewer than about 200 nucleotides) may be referred to as "oligonucleotides." Nucleic acid molecules are typically susceptible to degradation by nucleases.

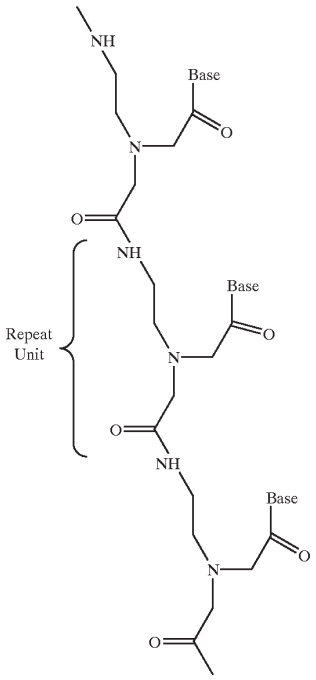
A "nucleobase" is a nitrogenous heterocyclic group typically found in nucleic acids (such as the purine bases adenine and guanine, or the pyrimidine bases cytosine, thymine and uracil), or an analog of such a group. Analogs include, for example, purine bases in which the ring substituents are other than those found in adenine or guanine, or pyrimidine bases in which the ring substituents are other than those found in uracil, thymine and cytosine. A number of analogs of nucleobases are well known in the art; many of which have been tested as chemotherapeutic agents. Some of these are described herein; see also, e.g., *Beilstein's Handbuch der Organischen Chemie* (Springer Verlag, Berlin), and Chemical Abstracts, which provide references to publications describing the properties and preparation of such compounds.

A "nucleobase polymer" is a polymer of nucleobases linked to a backbone. The backbone may be naturally occurring (as in a nucleic acid molecule) or may be non-naturally-occurring. Nucleobase polymers with non-naturally-occurring backbones are preferably resistant to degradative enzymes. Representative examples include peptide nucleic acids (see Buchardt et al., PCT WO 92/20702 and Buchardt et al., U.S. Pat. No. 5,719,262), morpholino-based nucleobase polymers (see Summerton and Weller, U.S. Pat. No. 5,698,685; Summerton et al., U.S. Pat. No. 5,378,841 and Summerton and Weller, U.S. Pat. No. 5,185, 444), peptide-base nucleic acid mimics or PENAMs (see Shah et al., U.S. Pat. No. 5,698,685), and polynucleosides with linkages comprising carbamate (see Stirchak and Summerton, *J. Org. Chem.* 52:4202, 1987), amide (see Lebreton et al., *Synlett. February* 1994:137), methylhydroxylamine (see Vasseur et al., *J. Am. Chem. Soc.* 114:4006, 1992), 3'-thioformacetal (see Jones et al., *J. Org. Chem.* 58:2983, 1993), sulfamate (see Huie and Trainor, U.S. Pat. No. 5,470,967) and others (see Swaminathan et al., U.S. Pat. No. 5,817,781 and Freier and Altmann, *Nucl. Acids Res.* 25:4429, 1997, and references cited therein).

"Particles" are discrete objects that when packed together yield a porosity ranging from 0.15 to 0.99, where porosity is defined as the fraction of the volume of the packed objects that is void space. Particles may have any shape, and may be, for example, spheres, cubes or irregularly shaped objects. Preferably the objects are substantially spherical (i.e., an object whose surface points are at a distance r±0.2 r from the object's center of mass), with a primary particle size ranging from 1 to 1000 Å. The choice of composition of the particles is such that the porosity is decreased less than 20%, and more preferably less than 5%, after contact with any agent to be employed, including irradiation, photoresist, developers, strippers and reagents. The percent decrease in porosity after contact with such agents can be readily assessed using nitrogen adsorption isotherms and the Brunauer-Emmett-Teller (BET) method. Other methods for assessing the percent loss in porosity after contact with process agents will be apparent to one of ordinary skill in the art.

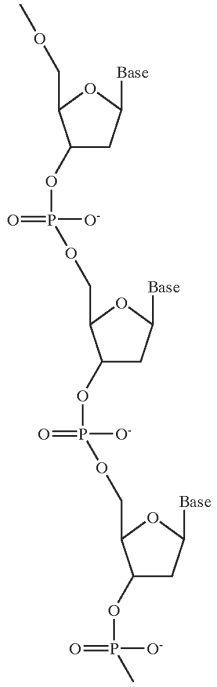
A "peptide nucleic acid" (PNA) is a molecule comprising repeating units of N-(2-aminoethyl)-glycine linked by amide bonds (see Buchardt et al., PCT WO 92/20702). Unlike the natural DNA backbone, no deoxyribose or phosphate groups are present. The bases are attached to the backbone by methylene carbonyl linkages.

PNA

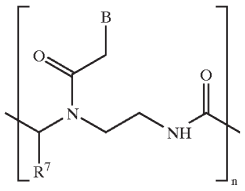


-continued

DNA

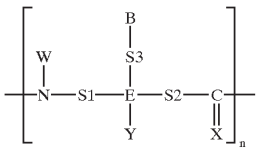


In this specification, PNA sequences are written using the single-letter designation of the attached base just as DNA sequences are written. PNA sequences are distinguished from DNA sequences by an "NH_2" group at what would be the 5' end of a DNA sequence. For example, in this specification AGGTC-5' is a DNA sequence, while AGGTC-NH_2 is a PNA sequence. Certain preferred peptide nucleic acid polymers comprise a repeating unit of the form:



wherein each B is independently selected from the group consisting of nucleobases; each R^7 is independently selected from the group consisting of hydrogen, C_{1-C8} alkylamines and spacers, and each n is an independently selected integer ranging from 1 to 100.

A "peptide nucleic acid mimic" (PENAM) is a nucleobase polymer that comprises a repeating unit of the form:



wherein each is E is independently selected from the group consisting of carbon and nitrogen; each W is independently selected from the group consisting of hydrogen and spacers; each Y is independently selected from the group consisting of hydrogen and spacers, in repeating units wherein E is carbon; each Y is a lone pair of electrons, in repeating units wherein E is nitrogen; each S1 is optional, and if present is an independently selected first spacer; each S2 is optional, and if present is an independently selected second spacer; each S3 is optional, and if present is an independently selected third spacer; each X is independently selected from the group consisting of oxygen and sulfur; each B is independently selected from the group consisting of nucleobases; N is nitrogen; and each n is an independently selected integer ranging from 1 to 100.

A "photocleavable moiety" is a portion of a molecule that is cleaved upon exposure to light of a particular wavelength and intensity.

"Photoresist" refers to a material that, upon irradiation, sustains a chemical reaction that allows irradiated and non-irradiated regions to be separated from one another. Although the separation may be simultaneous with the irradiation step (e.g., in laser ablation), it often requires an additional process step or steps (e.g., exposure to a developer). The chemical reaction may involve the formation or breakage of chemical bonds with such bond changes occurring in either an intramolecular or intermolecular fashion. In most applications, a photoresist is applied to a flat surface as a relatively thin liquid layer and evaporated. A "negative photoresist" refers to a photoresist that leaves photoresist on the surface in irradiated regions, while a "positive photoresist" refers to a photoresist that leaves photoresist on the surface in regions that were not irradiated. Certain positive photoresists comprise a base soluble component with phenolic hydroxyl groups. Within such photoresists, "base soluble" refers to a component with groups having a pKa of about 10 that are solubilized by aqueous solutions having a pH greater than about 10, and more preferably greater than about 11.

"Planarization" refers to a leveling process in a liquid layer applied to a substrate surface such that the free surface of the liquid layer is substantially planar despite irregular topography on the substrate surface.

A "polymer" is a molecule in which individual molecular units are repetitively linked by covalent bonds. A polymer of a hydrolyzed metal alkoxide comprises multiple hydrolyzed metal alkoxide molecules covalently linked to one another through oxane bonds.

A "polymerase" is an enzyme that catalyzes the assembly of ribonucleotides into RNA, or deoxyribonucleotides into DNA. "Polymerase chain reaction" (PCR) refers to a process for the exponential amplification of a specific DNA fragment using two oligonucleotide primers that hybridize to opposite strands and flank a region of interest in a target DNA (see Mullis, U.S. Pat. No. 4,683,202 and Mullis et al., U.S. Pat. No. 4,683,195). The process consists of a series of repetitive cycles involving template denaturation, primer annealing, and the extension of annealed primers by Taq DNA polymerase or other thermostable polymerase.

A coating is said to be "porous" if it contains void regions ranging from 1 to 1500 nm in diameter resulting in porosities ranging from 0.15 to 0.99, where porosity is defined as the fraction of the coating volume which has pores. For example, a porous coating of inorganic metal oxide particles contains void regions between inorganic metal oxide particles created by the packing of the metal oxide particles. Such a porous coating preferably has a "substantially uniform thickness" (i.e., the thickness of the coating varies by no more than 30% over the entire coated area). The average

pore size preferably ranges from 10 to 1000 nm, and may be readily determined by nitrogen adsorption isotherms and the Brunauer-Emmett-Teller (BET) method according to methods well known in the art. Within certain embodiments, the average pore size of a porous coating substantially approximates the particle size (i.e., the average pore size is p+0.9p, wherein p is the average particle size).

"Primary particle size" refers to the average size of unagglomerated single particles of inorganic metal oxide.

A "primer" is a nucleic acid or other nucleobase polymer designed to be sufficiently complementary to a target sequence in a denatured nucleic acid (in relation to its length) to be bound under selected stringency conditions so as to serve as a ligand for a polymerase. A primer should bind sufficiently to permit detection of the target sequence in a PCR assay.

A "probe" is a nucleic acid or other nucleobase polymer designed to be sufficiently complementary to a target sequence (in relation to its length) to be bound detectably under selected stringency conditions. A probe is typically labeled with a marker, such as a fluorescent moiety.

"Radiation" refers to energy which may be selectively applied, including energy having a wavelength of between 10^{-14} and 10^{4} meters. Radiation includes electrons, x-rays and particles from radioisotopic decay, as well as light (e.g., visible, ultraviolet or infrared).

A "reagent" is any compound that undergoes a chemical reaction with a molecule attached to a surface of an array. For example, a reagent may form a covalent bond with an attached molecule, permitting the synthesis of attached organic compounds using a series of reactions with known reagents.

"Reagent history" refers to a predefined sequence of reagents contacted with a predefined region of a solid-support. In most cases, the composition of a compound predicted by the reagent history and the actual predominant compound composition at a predefined region will be the same. However, the predicted composition will not accurately reflect the actual composition of the region in some embodiments. For example, when a reagent sequence comprises chemical reactions whose characteristics are not well defined, the predominant composition of a predefined region may not be predictable. In contrast, describing this predefined region by its reagent history uniquely defines the composition, which can be reproduced by the reagent history. Knowing the predominant composition of each array element is not always necessary in many applications. For example, a small-molecule array may contain an active drug candidate defined accurately only by its reagent history. Using this information, the candidate can be resynthesized on a large-scale, and the composition of the active component identified even if it is a minority fraction.

A "receptor" is a molecule that specifically binds a given ligand. Receptors may be naturally-occurring or man-made molecules, and can be employed in their unaltered state or as aggregates with other species. Receptors may covalently or non-covalently modify a given ligand after binding the ligand. Such modifications include labeling, altering conformation, cleaving, covalently binding and intercalation. A receptor that is capable of modifying a target ligand in such a manner is said to comprise a "target ligand modifying group." Examples of receptors include, but are not limited to, antibodies, cell membrane receptors, monoclonal antibodies, antisera reactive to specific antigenic determinants, enzymes, drugs, polynucleotides, nucleic acid, catalytic nucleic acids, peptides, catalytic peptides, peptide nucleic acids, morpholino-based nucleobase polymers, other nucleobase polymers, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes and organelles.

Compounds are "resistant to degradation by degradative enzymes" if less than 50% of the compounds are degraded after 10 minutes of contact with a degradative enzyme at a concentration equal to the K_m of the enzyme, and under conditions where the enzyme activity is known to be optimal (e.g., at an optimal temperature and salt concentration, and in the presence of optimal cofactors, prosthetic groups and coenzymes). Optimal conditions for a particular degradative enzyme will be readily apparent to those of ordinary skill in the art. The term "degraded" as used in this definition refers to one or more chemical alterations by the degradative enzyme that reduces the molecular weight of a compound. Degradative enzymes, within the context of the present invention, are naturally occurring nucleases and proteases. Representative degradative enzymes include, for example, specific and non-specific ribonucleases, deoxyribonucleases, exonucleases, and endonucleases, as well as specific and non-specific endoproteases and exoproteases. Numerous methods are available to those of skill in the art to test if a compound is resistant to degradation by degradative enzymes as defined above. For example, compounds may be contacted with degradative enzymes and the mixture subsequently subjected to an analytic procedure to determine if the molecular weight of greater than 50% of the compounds has been reduced. Such analytic procedures are numerous and will depend on the particular compound. They include, but are not limited to, high-pressure liquid chromatography (i.e., HPLC), gel electrophoresis, NMR spectroscopy, and IR spectroscopy. Other analytic procedures will be readily apparent to those skilled in the art. For example, HPLC may be used to detect the percent of a nucleobase polymer degraded by a non-specific single-strand deoxyribonuclease by dividing the integrated UV absorption of all chromatographic peaks other than the peak of the parent nucleobase polymer by the integrated UV absorption of all chromatographic peaks. Using such an analytic method, much more than 50% of an oligodeoxyribonucleotide will be degraded after 10 minutes of incubation with a non-specific single-strand deoxyribonuclease at a concentration equal to the enzyme's K_m. In contrast, nucleobase polymers possessing non-natural backbones will be degraded much less than 50% under identical conditions. As a practical matter, it is usually possible to identify a compound as resistant to a particular class of degradative enzymes by simply inspecting the chemical structure of the compound and determining if the structure differs appreciably from the natural substrate of the degradative enzyme. In particular, nucleobase polymers will be resistant to the general class of degradative enzymes known as nucleases if their backbone contains linkages other than the native phosphodiester linkage of nucleic acids. Similarly, nucleobase polymers will be resistant to the general class of degradative enzymes known as proteases if their backbone contains peptidic linkages comprising spacers or side-chains not found in proteins or peptides.

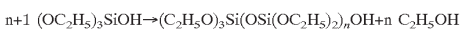
"Sol" refers to an intermediate in the "sol-gel" process. A sol is characterized by colloid-like oligomers formed from a chemical precursor.

"Sol-gel" refers to a method for preparing specialty metal oxide glasses and ceramics by hydrolyzing a chemical precursor or mixture of chemical precursors that pass sequentially through a solution (sol) state and a gel state before being dehydrated to a glass or ceramic. Preparation of metal oxide glasses by the sol-gel route proceeds through

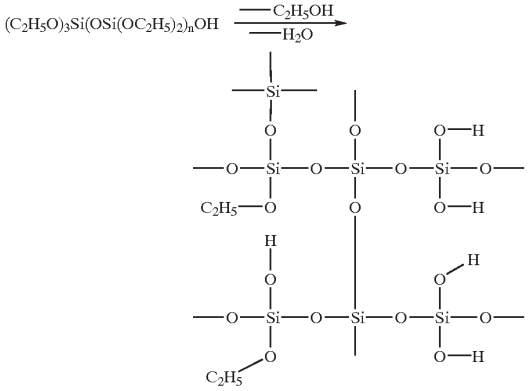
four basic steps: (1) partial hydrolysis of precursors to form reactive monomers; (2) polycondensation of these monomers to form colloid-like oligomers (sol formation); (3) additional hydrolysis to promote polymerization and cross-linking leading to a three-dimensional matrix (gel formation); and (4) further densification and cross-linking by drying and other dehydration methods. Although steps (1) through (3) are presented sequentially, after step (1) these reactions occur simultaneously to varying degrees. The chemical precursors are typically metal alkoxides, but may also include organo-metal alkoxides. A very common precursor is tetraethoxysilane, which proceeds through the sol-gel process according to the steps shown below:

(1) Monomer formation;

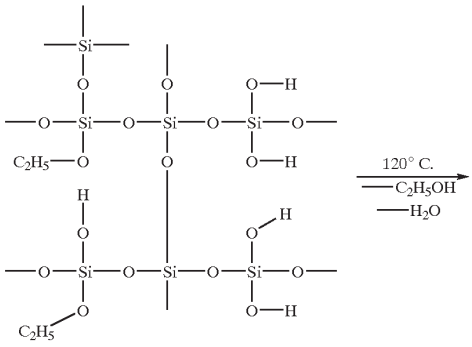
(2) Sol formation;



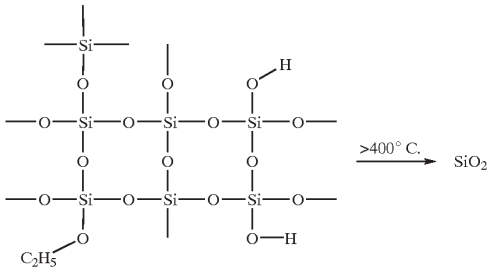
(3) Gelation; and



(4) Densification



-continued



Within certain embodiments described herein, coating solutions are formed that comprise substantially stable sots. The remaining steps of gelation and densification occur rapidly upon evaporation of solvent from an applied liquid layer of the sol. Curing at less than 250° C. results in "partial densification," with the network remaining relatively open with free silanols and some organic moieties still present. While not a necessary step in the present invention, very high temperatures do achieve the maximum density of silicon dioxide.

"Solvent Resistance" refers to the ability of a polymeric film to maintain integrity and impermeability while in contact with a particular solvent. A film is "solvent resistant" if contact with a particular solvent does not result in detectable cracking or dehiscence, or significant film dissolution, in the region where it is desired to place an array of compounds. Detectable cracks or dehiscence means cracks or dehiscence detected visually, or by light or phase-contrast microscopy. Significant film dissolution is defined as greater than 50% loss of film thickness after contacting the film with a particular solvent for a particular time period, and may be tested using profilometry or interferometry. It will be apparent that dehiscence, cracks, or loss of more than 50% of film thickness may be tolerated over regions where it is not desired to place an array of compounds. In some embodiments, the solvent resistance of a particular polymeric composition will be a function of film thickness. For instance, films which exceed a particular critical thickness will often crack in a particular solvent, presumably from solvent-induced stresses in the film that exceed the adhesive forces between the film and the substrate.

"Solution" refers to dispersions and suspensions of finely divided particles of ultramicroscopic size in a liquid medium, as well as the conventional definition of the term "solution." A "spacer" is a molecule that spaces an attached compound from a substrate. A spacer is relatively small, containing a backbone of 1–10 atoms (not counting hydrogen atoms), preferably selected from carbon, nitrogen, oxygen, and sulfur. Typically, such spacers comprise substituted or unsubstituted alkyl, alkenyl, alkenyl groups. However, spacers can also comprise for example: carbonyl (C═O), thiocarbonyl (C═S), amine (NH), substituted amine (NR), amide (C(═O)NH), substituted amide (C(═O)NR), carbamate (NHC(═O)O), urea (NHC(═O)NH), thioamide (C(═S)NH), substituted thioamide (C(═S)NR), hydrazine (NH—NH), substituted hydrazine (N(R)—N(R)), ether (C—O—C), thioether (C—S—C), disulfide (S—S), sulphone (S(═O)) and/or sulphoxide (SO_2) groups. A spacer can also be substituted with one or more small chemical groups, for example, small chain alk(ane, ene, yne)s, hydroxyl, alkoxyl, ketone, aldehyde, thiol, amino and/or halogen groups. A particular compound in an array may have multiple spacers, which may, but need not, be identical to one another. A spacer may also, or alternatively, be attached to one or more linkers.

"Stringency" refers to the combination of conditions by which complexes of aggregated nucleobase polymers (e.g., DNA:DNA, PNA:DNA or PNA:PNA) dissociate into individual component monomers. Common conditions used to influence stringency include pH, temperature, and salt concentration. See "T_m" below.

"Stripping" refers to the substantial removal of photoresist by strippers. Strippers are liquid chemical media used to remove photoresists after processing is finished. The exact composition depends on the composition of the photoresist.

"Substantial removal" of a photoresist from underlying molecules or porous coating means that photoresist is sufficiently removed to permit a desired reaction between underlying molecules or porous coating and a reagent. Such a reaction should have a yield that is at least 50%, and more preferably at least 90% of the yield observed for similar molecules that have not previously been coated with photoresist. Reaction yields may be readily determined with and without photoresist using standard techniques appropriate for the reaction of interest. Such techniques are well known to those in the art and include, for example, analysis of released protecting groups during synthesis as in the analysis of released trityl groups during solid-phase DNA synthesis, or analysis of free nucleophiles produced during synthesis as in the analysis of free amino groups during peptide synthesis using the ninhydrin reaction. Other methods include quantification of the final product while still attached to the substrate surface using, for instance, a surface acoustic wave sensor, or binding with a fluorescently labeled receptor (e.g., nucleobase polymer or antibody) and quantifying the fluorescent signals. Still other methods include releasing the final product from the substrate surface and quantifying it using high-pressure liquid chromatography, labeling with radioisotopes, and other methods familiar to those skilled in the art.

A compound is "substantially pure" if, within a discrete known region, the ligand is present at a concentration that is sufficient to permit the detection of distinguishing characteristics of the ligand. Such detection may be based, for example, on biological activity or function, which may be measured by way of binding with a selected ligand or receptor. Other characteristics that can be measured include, for example, color, light absorbance, light transmission, fluorescence, phosphorescence, molecular weight, charge, density, melting point, chromatographic mobility, turbidity in a solution (i.e., nephelometry), electrophoretic mobility, mass spectrum, ultraviolet spectrum, infrared spectnum, nuclear magnetic resonance spectrum, elemental composition, and x-ray diffraction. Preferably, a substantially pure ligand is present in a region at a level that is greater than 5%, 10%, 50%, 70% or 90% of the total compounds with the region.

"Surface density" refers to the number of molecules contained in a three-dimensional volume projected on a two-dimensional space. For example, 1000 molecules in a volume with dimensions x=10 μm, y=10 μm and z=1 μm would have a surface density in the x-y space of 1000 molecules per 100 μm^2 or 10 molecules/μm^2. The surface density in the x-z space would be 1000 molecules per 10 μm^2 or 100 molecules/μm^2. If z=3 μm, the surface density in the x-y space would be 300 molecules/μm^2. When discussing ligand-arrays, the molecules are ligands and the projected space is taken to be equivalent to the largest planar component of the substrate.

A "substantially uniform layer" is a layer that varies in thickness by no more than 30% over a region of interest.

"T_m" refers to the temperature at which two complementary polymers dissociate into individual nucleobase components. An approximate value of T_m for a DNA duplex in degrees centigrade is given by the formula:

$$T_m = 16.6 \log[M] + 0.41[P_{gc}] + 81.5 - P_m - B/L$$

where M is the molar concentration of Na^+ to a maximum of 0.5, P_{gc} is the percent of G or C bases in the oligonucleotide between 30% and 70%, P_m, is the percent mismatch, B is 675 for oligonucleotides less than 100 bases, and L is the probe length in bases. For a PNA:DNA heteroduplex in 100 mM NaCl, the T^m is approximately 1° C. higher per base pair than the corresponding DNA duplex.

Coated Articles

The present invention provides articles comprising a continuous porous coating to which one or more compounds may be attached. In general, such porous coatings have a substantially uniform thickness, and comprise a gelled network of particles. The porous coatings may also be patterned using a photoresist and photolithographic methods in a fashion which allows exemplary reproducibility and control over the dimensional features of the patterned porous coating.

The present invention has a variety of uses including, for example, applications which require the synthesis of a large number of known compounds at known locations on a solid support, each in quantities sufficient for diagnostics or pharmacologic screening. Such applications exist in the field of array manufacturing where microfabrication and solid-phase synthetic methods presently result in only low ligand surface densities. The instant invention overcomes the limitations associated with imaging ligand-receptor binding on low ligand surface densities by providing a support with increased surface area for ligand attachment. Using a porous coating provided herein, imaging may be accomplished rapidly and with less costly equipment. The invention also eliminates the need for other equipment, such as a flow-through apparatus to contact reagents or receptors with arrayed ligands.

The increased surface density of drug candidates on a porous array, as described herein, permits the performance of functional assays using a single array element. Furthermore, drug candidates that have only weak to moderate binding may be identified during screening because the increased surface density ultimately provides a higher assay concentration of the candidate. Similarly, arrays of small-molecules bound on the porous support of the present invention can be used to screen for other activities including pesticide or herbicide activities.

Briefly, articles comprising porous coatings as provided herein may be prepared by: (a) applying to a substrate a substantially uniform layer of a solution comprising particles dispersed in a volatile liquid; (b) evaporating the volatile liquid from the layer, forming a gelled network of particles on the substrate; and (c) attaching two or more compounds to discrete known regions of the porous coating. Various modifications may be made to this method, including using a polymer-particle composite to form the gelled network, photopatterning the porous coating and enhancing porous coating strength through the use of a fortifying layer and/or a curing step. Each of these steps is described in greater detail below.

A. Substrate Selection and Preparation

Nearly any conceivable substrate may be employed, including substrates that are biological, nonbiological, organic, inorganic or a combination of any of these. The substrate may have any convenient shape, such as a disc, square, sphere, circle, or any other suitable shape, and may be formed, for example, as a particle, strand, precipitate, gel,

sheet, tube, sphere, container, capillary, pad, slice, film, plate or slide. The substrate should form a rigid support on which to support the porous coatings described herein, and is preferably flat, although it may have a variety of alternative surface configurations, including raised and/or depressed regions. The substrate may be prepared from essentially any material. For instance, a substrate may comprise functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SIN_4, modified silicon, photoresist, biolayers, silane layers or any one of a wide variety of polymers such as polytetrafluoroethylene, polyvinylidenedifluoride, polystyrene, polycarbonate, polyethylene, polypropylene, nylon, polyethylene terephthalate or combinations thereof. Other substrate materials will be readily apparent to those of skill in the art. In a preferred embodiment the substrate is flat glass or single-crystal silicon.

The surface of a substrate may, but need not, be composed of the same material as the substrate. Surface materials include, but are not limited to, polymers, plastics, resins, polysaccharides, alumina, silica or silica-based materials, carbon, metals, inorganic glasses, membranes or any of the above-listed substrate materials. Preferably, the surface contains reactive groups, such as carboxyl, amino and/or hydroxyl groups. Most preferably, the surface will be optically transparent and will have surface Si—OH functionalities, such as are found on silica surfaces. Surfaces are also preferably rigid.

Optionally, a surface of a substrate may have an adhesive layer, which is stably attached to the substrate and promotes adhesion of the porous coating to the substrate. It will be apparent that any of a variety of adhesive layers may be used. In preferred embodiments, an adhesive layer comprises partially or substantially hydrolyzed monomers, oligomers, and/or extended polymers of a metal alkoxide or organo-metal alkoxide coupled to one another through "oxane" bonds (ie., "-M-O-M-"; condensation products of: -M-OH+OH-M-→-M-O-M-+H_2O, where M is a metal). For example, the adhesive layer may comprise one or more polymers of a hydrolyzed organo-metal alkoxide of the formula: $R_{n'M(OR)x}$, wherein M is selected from the group consisting of Si, Ti, Al, B, Zr, Er, Cr, Ga, Ge, Hf, Fe, Ca, Cr, La, Mg, Nb, K, Pr, Sm, Na, Ta, Te, Tl, Sn, W, V, Y and Zn; R' is a monovalent organic group containing between 1 and 12 carbon atoms, preferably having one or more groups selected from the group consisting of hydrogen, amine, hydroxyl, carboxyl, amide, thiol, sulfonic, or epoxide; R is hydrogen, an alkyl group or an aryl group and n and x are integers independently selected from the group consisting of 0, 1, 2, 3 and 4. A sol may be prepared from such a metal alkoxide by hydrolyzing and aging the metal alkoxide at an acidic pH, as described below. In a preferred embodiment, the adhesive layer comprises a polymer of hydrolyzed tetraethoxysilane (e.g., the layer may be a partially densified layer of partially or substantially hydrolyzed tetraethyoxysilane). The adhesive layer is of sufficient thickness to provide stable attachment between a porous coating and a substrate. In some embodiments the thickness of the adhesive layer is less than 0.001 μm, 0.01 μm, 0.1 μm, 1.0 μm, or 10.0 μm. Preferably, the thickness is between 0.002 and 2 μm, more preferably between 0.1 μm and 10.0 μm.

An adhesive layer may be formed on a substrate by applying a substantially uniform layer of an adhesive solution (comprising a metal oxide or organo-metal alkoxide polymer and a volatile solvent) to the surface of the substrate, and evaporating the volatile solvent. The solution may contain various solvents or mixtures of solvents, in which the partially or substantially hydrolyzed monomers and condensation products of the organo-metal alkoxide are soluble. For example, suitable solvents include ethylene glycol monomethyl ether, ethyl alcohol, methyl alcohol, butyl alcohol water and mixtures thereof, in such proportions as to give desirable evaporation characteristics. Preferred adhesive solutions comprise an aged sol of hydrolyzed tetraethoxysilane in a volatile solvent. One preferred sol comprises 1.5 volume percent tetraethoxysilane, in 0.45 volume percent water, 3 mM nitric acid, with the balance ethanol. A concentrated sol may be prepared by hydrolyzing 21.7 ml of tetraethoxysilane in 6.3 ml H_2O and 0.7 ml 1N nitric acid, followed by aging at 4° C. for several days. The concentrated sol is clear and stable for several weeks at 4° C. An adhesive solution may be applied to a surface by any suitable technique, including dip-coating, spin-coating or microdispensing. In one embodiment, the concentrated sol described above is diluted 50-fold with ethanol, and applied to surface at an incline using a pipette.

After the adhesive solution is applied as a liquid layer, the solvent is allowed to evaporate, resulting in an adhesive layer. Evaporation may take place at any suitable temperature between 10° C. and 150° C., and most preferably at room temperature. Following evaporation, an adhesive layer may be cured to enhance adhesion. Curing may take place, for example, at a temperature that ranges from 20° C. to 250° C. for a period of time sufficient to establish an extensive network of oxane bonds. In general, about 15 minutes at 120° C. is sufficient. The final thickness of an adhesive layer may be controlled by altering the percent solids in the sol, molecular weight of the solids, viscosity, incline angle, withdrawal-rate in the case of dip-coating, or the spin speed in the case of spin-coating.

B. Formation of Gelled Network of Particles

As noted above, porous coatings as described herein comprise a gelled network of particles. Such a gelled network may generally be prepared by applying to the substrate a substantially uniform layer of a solution comprising suitable particles (and, optionally, a polymeric component) dispersed in a volatile liquid. Following application, the volatile liquid is evaporated, forming a porous coating that comprises a gelled network of particles.

There are a variety of particles that may be used to form a porous coating. Such particles may comprise, for example, one or more of: carbon, activated carbon, fluorinated carbon, styrenedivinylbenzene copolymers, polystyrene, zeolites, oxides of antimony and oxides of metals present within Group III and Group IV of the Periodic Table. The selection of the particle composition is dependent upon the ultimate balance of properties desired, and upon whether formation of the gelled network depends on interaction between particles (or, as described below, is facilitated through the use of a polymeric component). Particles may have any of a variety of sizes and shapes. Preferably, the particles have a primary particle size of less than 2000 Å, and more preferably less than 1000 Å and still more preferably less than 500 Å. In some embodiments, particles have a primary particle size less than 100 Å, 50 Å, 10 Å or 5 Å. Although in most embodiments, a porous coating will be prepared with metal oxide particles having a narrow size distribution, it may be desirable in some embodiments to use particles with a broad size distribution (e.g., by mixing particles of different primary particle sizes). The particles are preferably spherical, although other shapes are possible, including cubes and irregular shapes.

Within certain preferred embodiments, the particles are metal oxide particles. Metal oxide particles particularly suitable for use in preparing a porous coating are those in

which the metal oxide particles are negatively charged. Such particles include, but are not limited to, particles comprising tin oxide (SnO_2), titania, antimony oxide (Sb_2O_5), silica, silicalite, fumed silica, alumina and alumina-coated silica as well as other metal oxides of Groups III and IV of the Periodic Table and mixtures thereof. Particularly preferred particles include silica and alumina-coated silica particles. In one embodiment, the metal oxide particles are spherical fumed-silica (SiO_2) particles with a primary particle size of 500 Å.

Certain particles are particularly suited for preparing porous coatings in the absence of a polymeric component. For example, porous coatings may be formed from strongly branched particles formed by sol-gel methods that bond to one another through oxane bonds (see Brinker et al., *Thin Solid Films* 201:97, 1991 and Brinker et al., in: *Ultrastructure Processing of Advanced Materials*, Wiley-Interscience, John Wiley and Sons, edited by Uhlmann and Ulrich, (1992), p. 211). Alternatively, porous coatings may be formed from both aged and unaged colloidal silica solutions directly (see Frye et al., in: *Better Ceramics Through Chemistry IV*, vol. 180, Mat. Res. Soc. Symp. Proc., edited by Brinker et al., (1990), p. 583; Frye et al., U.S. Pat. No. 5,224,972; Frye et al., U.S. Pat. No. 5,589,396, Cathro et al., *Solar Energy* 32:573, 1984 and Lange et al., U.S. Pat. No. 4,816,333).

Within certain embodiments, the gelled network further comprises a polymer suitable for forming a polymer-particle composite. Polymers of hydrolyzed metal alkoxides are preferred. Such polymers may be various condensation products (a small fraction, e.g., less than 20 weight percent, of the metal alkoxide may exist as monomer) of a hydrolyzed metal alkoxide of the formula $M(OR)_x$, where x may be 3 or 4; and R is hydrogen, an alkyl group or an aryl group (e.g., methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tertiary butyl group, or mixtures thereof). The metal, M, may be Si, Ti, Al, B, Zr, Er, Cr, Ga, Ge, Hf, Fe, Ca, Cr, La, Mg, Nb, K, Pr, Sm, Na, Ta, Te, Tl, Sn, W, V, Y or Zn. In one embodiment, the metal alkoxide polymer comprises a partially densified layer of hydrolyzed tetraethyoxysilane. The ratio of metal oxide particles to metal alkoxide polymer should be in a range which adequately binds the particles together and to the adhesive surface, and provides a substantially uniform coating, but does not result in metal alkoxide polymer substantially filling the pore volume. In preferred embodiments, in which silica particles are employed, the weight ratio of silica particles to hydrolyzed metal alkoxide ranges from 1–1000:1, preferably 3–1000:1, and more preferably 40–350:1.

It will be apparent that other polymer-particle composites are possible. For example, in some embodiments porous coatings may be polymer-particle composites of metal oxide-hydroxide particles linked by cellulosic polymers (see Farooq et al., U.S. Pat. No. 5,686,602 and Desu et al., PCT WO 94/14088). Suitable cellulosic polymers include, for example, hydroxypropyl methylcellulose, methylcellulose, hydroxypropyl ethylcellulose and hydroxyethyl cellulose. The cellulosic polymers may be either left in the porous coating as a polymer cross-linked by metal ions, or removed by sintering. Another suitable polymer that may be sintered is polyimide (Desu et al., PCT WO 94/14088). In other embodiments the polymer-particle composite comprises alumina particles covalently linked by a photosensitized polyethyleneimine, or colloidal silica particles linked by an epoxide (e.g., glycidoxypropyltrimethoxysilane) and a polyamine (e.g., triethylene tetramine) (see Pu et al., *J. Imaging Sci.* 33:177, 1989 and Chu et al., *Mat. Res. Soc. Symp. Proc.* 435:221, 1996). In still other embodiments the polymer-particle composite comprises particles noncovalently linked by polytetrafluoroethylene wherein the particles may be comprised of finely divided carbon, activated carbon, fluorinated carbon, alumina, silica, silicalite (described by Grose et al., U.S. Pat. No. 4,061,724), fumed silica, styrenedivinylbenzene copolymers, polystyrene, zeolites, and various metal oxide particles (see Suppiah, U.S. Pat. No. 5,120,600).

It should be noted that organo-metal alkoxides such as APES and HAPES, do not function as substitutes for metal alkoxides when the particles are fumed silica. Hydrolysis of APES and HAPES results in the formation of organosilsesquioxanes, which form considerably less extended polymers than metal alkoxides due in part to stabilization of silanols through internal hydrogen bonding with the amino moiety. The lower degree of APES and HAPES polymerization may explain their ineffectiveness.

For preparation of a porous coating, the particles (and polymeric component, if desired) are combined with a suitable solvent to form a solution. It will be apparent that such a solution may be a dispersion or suspension of finely divided particles. The coating solution may contain various solvents or mixtures thereof in which the various polymer components are soluble, and in which the particles may be effectively dispersed or suspended. Suitable solvents will be apparent to those of ordinary skill in the art. For example, solvents may be used such as ethylene glycol monomethyl ether, 3-pentanone, ethyl alcohol, methyl alcohol, butyl alcohol, water, or mixtures thereof, in such proportions as to give desirable evaporation characteristics. In preferred embodiments, in which a coating solution comprises metal oxide particles and condensation products of a metal alkoxide polymer, the coating solution may contain, for example, about 93% ethanol and 7% H_2O. Such coating solutions preferably contain about 0.2 to 25 weight percent, more preferably about 2 to 10 weight percent, metal oxide particles.

Standard techniques may be employed to obtain condensation products of a metal alkoxide polymer. Briefly, the polymer may be partially hydrolyzed (ie., from 10% to 50% of hydrolyzable bonds hydrolyzed) or substantially hydrolyzed (i.e., greater than 50% of hydrolyzable bonds hydrolyzed) by exposure to a suitable amount of water. The hydrolyzed polymer then forms condensation products, which result in extended polymers (see glossary) through the formation of oxane bonds. Such polymers may be formed before or after addition of metal oxide particles. The process of forming extended polymers is referred to herein as aging. While a variety of conditions may be used for this process, it has been found that aging for at least one day at an acidic pH is usually sufficient. The acidity of the coating solution is preferably between 2.0 and 5.0 pH units, and most preferably between 4.0 and 5.0 pH units. Increasing the acidity beyond about 7.0 pH units results in solution instability due to polymer aggregation. In contrast, coating solutions formed at an acidic pH are stable for at least 6 months at 4° C.

Within a preferred embodiment, a porous coating may be generated using a metal alkoxide and fumed silica particles. The concentration of metal alkoxide is preferably 20 μmole to 2000 μmole, and more preferably 60 μmole to 240 μmole, per gram of 500 Å silica particles. A coating solution may be made by mixing 50.0 ml of 5 weight percent silica particles (500 Å primary particle size) dispersed in 95% ethanol/5% H_2O, 0.435 ml of 6 mM HNO_3, and 0.100 ml of tetraethyoxysilane (180 μmole/g of silica particles). The coating

solution thus formed may be mixed in a plastic container at room temperature for greater than 24 hours, and preferably greater than 48 hours. Optionally, the coating solution may be sonicated before use.

The coating solution may be applied using any standard technique, including dip-coating, spin-coating or microdispensing. According to one embodiment, the coating solution is applied to a substrate at an incline using a pipette. As noted above, the substrate on which the porous coating is prepared may, but need not, comprise an adhesive layer to enhance adhesion of the porous coating.

After the coating solution is applied, the solvent is allowed to evaporate leaving a continuous porous coating that is substantially uniform in thickness, and comprises a gelled network of substantially spherical metal oxide particles, and polymers of a hydrolyzed metal alkoxide coupled to one another through oxane bonds. In preferred embodiments, the evaporation of the solvent may be performed at a temperature between 10° C. and 150° C. Solvent is most preferably evaporated at room temperature.

The resulting porous coating is continuous, rigid, substantially uniform in thickness, and comprises a gelled network of particles. The metal oxide particles (and the metal alkoxide polymer, if used) are bound to one another and the substrate through oxane bonds. Particles may further be linked to one another by noncovalent bonds.

The final thickness of the porous coating may be controlled by altering the percent solids, viscosity, incline angle, withdrawal-rate in the case of dip-coating, or the spin speed in the case of spin-coating. The thickness of the porous coating may vary over a wide range (e.g., 0.05 to 25 microns). Such a coating preferably has a surface area that is at least 50 meters^2/g, or at least 100 square microns per cubic micron of porous coating.

A porous coating as described herein provides a high surface area for ligand attachment, thus increasing ligand density. The surface area is inversely proportional to average pore size. In turn, the average pore size closely approximates the primary particle size. Thus, the surface area and the average pore size of the coating may be tailored by the choice of primary particle size (i.e., the coating has controlled porosity) For example, a metal oxide with a primary particle size of 500 Å will have a surface area of 50 m^2/g, and a micron thick coating of such particles will increase the ligand density 100-fold. Similarly, a metal oxide with a primary particle size of 200 Å will have a surface area of 200 m^2/g, and a micron thick coating will increase ligand density 400-fold. In contrast, a primary particle size greater than 1000 Å yields porous coatings with surface areas too small to be useful in the present invention. In preferred embodiments, surface area is optimized by choosing a primary particle size not larger than that required for a given application of the invention. For example, in applications requiring solid-phase synthesis or detection of ligand-receptor binding, the surface area is optimized by choosing a primary particle size not larger than that required for sterically unrestricted diffusion of molecules through the porous network. In particular, molecules measuring less than about 10 Å, 50 Å, 100 Å, 200 Å, 500 Å, and 1000 Å will gain access to the interior of the porous coating if the coating is comprised of particles measuring greater than about 10 Å, 50 Å, 100 Å, 200 Å, 500 Å, and 1000 Å in diameter, respectively.

The surface area of a porous coating may be further increased by increasing the thickness of the coating. In the instant invention, is has been discovered that a crack-free coating up to 25 μm thick is possible with a single application of the above dispersion. In some embodiments, the porous coating is less than 1.0 μm, 2.0 μm, 5.0 μm, 10.0 μm or 25.0 μm thick. In general, however, adequate surface area enhancements are obtained when the thickness is between 2.0 μm and 5.0 μm.

D. Photopatterning

An article may comprise a single porous coating, or multiple discrete porous coatings. An article having multiple coatings may be prepared by photopatterning using photolithographic methods. Briefly, a porous coating may be applied to a substrate as described above. A layer of photoresist (which may be positive or negative) may then be established over the porous coating, sufficient to substantially cover the porous coating and fill its pore volume. Using a mask or other suitable irradiation-targeting device, the photoresist is irradiated at one or more discrete regions, such that subsequent contact of the photoresist with developer results in dissolution of the photoresist and the porous coating underlying it at one or more discrete regions. When a positive photoresist is used, contact with developer results in removal of irradiated regions of photoresist, and underlying porous coating. When a negative photoresist is used, contact with developer results in removal of the regions that were not irradiated. Stripping of the remaining photoresist with an organic solvent yields separate porous coatings on the substrate. The resulting article preferably comprises greater than 10^3, 10^4, 10^5 or 10^6 porous coatings, and each coating preferably has an area between about 1 cm^2 and 10^{-12} cm^2. In some embodiments, the area occupied may be extremely small, being limited by the size of the individual metal oxide particles. For example, a porous coating may occupy an area less than about 10^{-1} cm^2, 10^{-2} cm^2, 10^{-3} cm^2, 10^{-4} cm^2, 10^{-5} cm^2, 10^{-6} cm^2, or 10^8 cm^2, or 10^{-12} cm^2. In preferred embodiments, the area occupied by each porous coating is preferably between about 1 μm^2 and 1 mm^2, more preferably less than about 10,000 μm^2, and still more preferably less than 100 μm^2. The methods provided herein provide exemplary reproducibility and dimensional control consistent with the mass production of patterned porous coatings with micron-scale features.

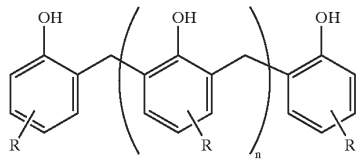
Each of the separate porous coatings, as well as their group arrangement, can assume essentially any size and any shape. For example, squares, ellipsoids, rectangles, triangles, circles, or portions thereof, along with irregular geometric shapes, may be utilized. Duplicate arrangements (e.g., more than 100) may also be applied to a single substrate for purposes of redundancy. In a preferred embodiment, the separate porous coatings are arranged as an array. As described in more detail below, such an array may be used as a ligand-array, with each porous coating comprising substantially pure ligands with a known and unique chemical composition.

1. Application of Photoresist

The photoresist layer preferably comprises a component that is base-soluble (either before or after irradiation). Many imaging chemistries are known in the art that utilize a radiation-induced change in base-solubility of a polymer (see *Desk Reference of Functional Polymers: Synthesis and Applications*, edited by Reza Arshady, (1997), American Chemical Society, Washington, D.C., pages. 295, 301, 320–326, and 341–366). Such imaging chemistries have been used to produce both positive and negative photoresists.

Certain preferred photoresists comprise a component with attached phenol groups, such as a phenolic polymer obtained

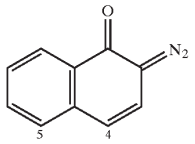
by the step polymerization of phenol and formaldehyde of the general form:



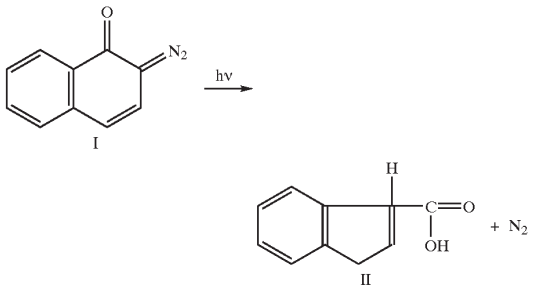
where n=0–13 and R═H or alkyl. Phenolic polymers are soluble in basic aqueous solutions through the formation of phenolate ions. For example, in some embodiments, a positive photoresist may be used that combines a phenolic polymer with a dissolution inhibitor such as a diazoquinone, onium salt, α-diazoacetoacetate, or o-nitrobenzyl cholate. Alternatively, a negative photoresist may be used that combines a phenolic polymer with a cross-linking agent such as a diazoquinone, a bisazide, or acid-activated agent in combination with a photoacid generator. Negative photoresists may also be used that are produced through masking of the base-solubilizing OH functionality of the phenolic polymer by photochemical esterification with, for example, 1,3-dioxin-4-one, diphenyltetrazole, or a polyhalide. In still other embodiments, the photoresist may be a chemically-amplified photoresist produced by combining a photoacid generator such as an onium salt, nitrobenzyl ester, or imino sulfonate with a phenolic component. Such a phenolic component may be formed from a phenolic polymer blended with an acid-labile dissolution inhibitor, a phenolic polymer capable of acid-catalyzed depolymerization, or a phenolic polymer derivatized at the OH functionality with acid-labile groups such as tert-butoxycarbonyl (t-Boc), benzhydryloxy-carbonyl (Bhoc), trimethylsilyl, t-butyl, phenoxyethyl, or tetrahydropyranyl. Other positive and negative photoresists containing phenolic polymers will be apparent to those of skill in the art.

According to a preferred embodiment, the photoresist layer is from the large class of commercially available positive photoresists comprising a phenolic polymer and a diazoquinone dissolution inhibitor (see U.S. Pat. Nos. Steinhoff et al., 3,402,044; Moore, U.S. Pat. No. 2,797,213; Endermann et al, U.S. Pat. No. 3,148,983; Schmidt, U.S. Pat. No. 3,046,118; Neugebauer et al., U.S. Pat. No. 3,201,239; Sus. U.S. Pat. No. 3,046,120; Fritz et al., U.S. Pat. No. 3,184,310; Borden, U.S. Pat. No. 3,567,453; and Pampaione, U.S. Pat. No. 4,550,069). Such positive photoresists are typically prepared in a liquid form comprising 10 to 40 weight percent phenolic polymer, 10 to 40 weight percent diazoquinone, and an organic solvent such as 2-ethoxyethyl acetate or 1-methoxy-2-propyl acetate. Other additives, such as surfactants, may be present in minority fractions to promote planarization of the photoresist. In preferred embodiments, a photoresist layer is derived from such a positive diazoquinone photoresist applied to the porous coating as a thin liquid layer. Most preferably, the liquid photoresist is AZ® 1500 series positive photoresist manufactured by Hoechst TM Celanese, Somerville, N.J.

The photolytic response of phenolic photoresists reflects the photochemistry of the photosensitive diazoquinone often also referred to as a diazoketone, diazo-oxide, diazoanhydride, or quinone diazide, a chemical of the general form:



where the most commonly used versions of the general form are substituted at positions 4 or 5 with an —SO_2R group, where R consists of a very large variety of functionalities including sulfonic acid esters and amides of both monomeric and polymeric hydroxy, phenoxy, and amino compounds well described in the patent literature and familiar to those skilled in the art (described extensively in DeForest, Photoresist Materials and Processes, McGraw-Hill (1975)). The primary photochemical behavior of the diazoquinone is substantially the same regardless of the composition of R. Exposure to radiation with wavelengths from about 220 nm to 450 nm leads to the photolytic conversion of the base-insoluble diazoquinone (I) to an acid species (II) soluble in aqueous base.



This conversion leads to a differential solubility between irradiated and non-irradiated photoresist, with irradiated regions soluble in aqueous base and non-irradiated regions substantially insoluble. Aqueous base is used herein as a developer for base-soluble photoresists, as described in greater detail below. The photolytic conversion occurs with high photosensitivity. For example, the photospeed of AZ® 1512 positive photoresist is 58 mJ/cm^2.

Photoresist may be applied using any standard technique. For example, a liquid photoresist may be applied as a thin liquid layer with a pipette. Excess photoresist may be allowed to drain by positioning the porous coating at an incline. Alternative methods of liquid photoresist application will be apparent to those skilled in the art, including dip-coating, spin-coating and microdispensing. All operations in the process of applying, irradiating and developing a photoresist should be carried out in a room lit primarily or entirely by light of a wavelength outside of the light range which will react with the photoresist. This may be accomplished with a protective golden shield or sleeve that blocks light less than 505 nm, placed over standard cool-white fluorescent lights (Imtec Products Inc., Sunnyvale, Calif.).

After a photoresist solution is layered onto the porous coating, the photoresist layer may be generated by evaporating the solvent. During this evaporation step, prolonged exposure to temperatures greater than about 100° C. is avoided. For example, evaporation of solvent from the coating solution is performed at a temperature less than 90°

C. (such as 85° C. to 90° C.), 50° C., 30° C., or 10° C. Most preferably, the solvent is evaporated at room temperature.

Following application, the photoresist should be continuous and cover any underlying porous coating. More specifically, the porous coating should reside under a layer of photoresist from 0.1 to 20 microns thick, preferably 0.2 μm to 4.0 μm thick and more preferably 1 to 3 microns thick. It should be noted, however, that thicker photoresists (e.g., greater than 25 microns) may be used. The final thickness of the photoresist layer may be controlled by altering the percent solids in the liquid photoresist, the molecular weight of the solids, the viscosity, incline angle, withdrawal-rate in the case of dip-coating, or the spin speed in the case of spin-coating. Depending on the thickness of the photoresist, the surface of the photoresist will be flat or will follow the surface contour of the porous coating and raised and/or depressed regions or elements. In general, the surface contour of the photoresist will be at least 0.1 microns higher than the surface contour of the porous coating(s).

2. Irradiation

Irradiation of a photoresist layer with a specific wavelength of light permits the selective, substantial removal of photoresist from irradiated (positive photoresists) or non-irradiated (negative photoresists) regions. This property results from differential solubility of irradiated photoresist, as compared to non-irradiated photoresist. The extent of this differential solubility may be assessed by exposing a selectively irradiated photoresist layer to developer and assessing the extent to which photoresist has been removed from irradiated and non-irradiated regions (e.g., using profilometry). In general, a differential solubility of at least 20-fold is sufficient. For example, irradiation of a positive photoresist and exposure to developer resulting in removal of at least 2 microns of a photoresist in irradiated regions, should result in the removal of no more than 0.1 microns in non-irradiated regions, as determined by profilometry.

Although the photoresist is preferably reactive to radiation that is in the visible, near-UV, mid-UV, or deep-UV portions of the electromagnetic spectrum, depending on the photoactive species, the photoresist may also be reactive to infrared, electron beam, x-ray or any other radiation. In some embodiments, it may be desirable to utilize photoresists sensitive to different wavelengths of light so as to, for example, selectively photopattern one of two photoresists located in different regions by irradiating both regions simultaneously.

The photoresist layer is selectively irradiated (i.e., a portion of the photoresist is irradiated with a wavelength that alters the solubility of the irradiated region). Such selective irradiation may be achieved using one or more masks and photolithographic techniques of the type known in the semiconductor industry (see Sze, VLSI Technology, McGraw-Hill (1983), and Mead et al., Introduction to VLSI Systems, Addison-Wesley (1980)). Light is preferably directed at the surface layered with the photoresist, but may also be directed at the back of the substrate, so long as it is transparent to the wavelength of light needed to react with the photoresist. The photoresist may be irradiated either in contact or not in contact with a solution, and is preferably irradiated not in contact with a solution. Using the photolithographic methods disclosed herein, it is possible to mask light to very small and precisely known locations, thereby achieving a method with exemplary reproducibility and dimensional control consistent with the production of porous coatings and porous coatings bearing ligand-arrays with micron-scale features.

A mask employed for the selective irradiation is generally an opaque support with transparent regions that allow the free passage of light to selected regions of the photoresist. Opaque regions may block light by absorbing or reflecting it. Within preferred embodiments, an ordered sequence of masks is used. In some embodiments it is possible to minimize the number of masks by utilizing the same mask to irradiate different regions by translating and/or rotating the mask with respect to each of the regions. A mask may be, for example, a glass sheet having etched chrome thereon or a silver-halide film with opaque regions obtained by laser-photoplotting. Such masks are manufactured by, for example, Precision Image Corporation, Redmond, Wash.

The transparent regions of a mask are in a pattern substantially identical to the pattern of light that will irradiate the photoresist layer, and permit the passage of light in a pattern that corresponds to the irradiated regions. The transparent regions may be of any size or shape. For example, squares, ellipsoids, rectangles, triangles, circles, or portions thereof, along with irregular geometric shapes, may be utilized. In preferred embodiments, the area of each transparent region is extremely small being between about 1 cm^2 and 10^{-12} cm^2, preferably less than 0.3 cm^2, and most preferably between about 1 μm^2 and 1 mm^2. For example, a transparent region may have an area less than about 10^{-1} cm^2, 10^{-2} cm^2, 10^{-3} cm^2, 10^{-4} cm^2, 10^{-5} cm^2, 10^{-6} cm^2, 10^{-7} cm^2 or 10^{-8} cm^2. In preferred embodiments, a mask comprises a plurality of transparent regions. In some embodiments, a mask comprises more than 10^2, 10^3, 10^4, 10^5, 10^6, 10^8 or 10^9 separate transparent regions. In preferred embodiments, a mask comprises greater than 100 duplicates of an array of separate square or circular transparent regions, each array comprising greater than 10^3, 10^4, 10^5 or 10^6 transparent regions. It will be understood, of course, that the irradiated regions of a photoresist layer will have sizes, shapes and numbers substantially identical to the transparent regions of the mask.

During irradiation, a mask is brought into close proximity with, imaged on, or preferably brought directly into contact with the photoresist surface. In alternative embodiments, the mask may be some distance away from the photoresist surface, as occurs in the technique known as projection printing. Alignment may be performed using conventional alignment techniques in which alignment marks are used to accurately overlay successive masks, or more sophisticated techniques may be used. For example, interferometric techniques may be used (see Flanders, *App. Phys. Lett.* 31:426, 1977). In some embodiments, a patterned porous coating may itself serve as an alignment mark.

With the mask appropriately positioned over the photoresist, the mask is irradiated with light. The light may be from a conventional incandescent source, a UV source, a laser, a laser diode, an excimer laser, an x-ray source, a programmable mask, a fiber optic or the like. In some embodiments, a positive photoresist layer may be irradiated with 365 nm light from a UV transilluminator manufactured by UVP Inc. (Upland, CA) at an energy density of 8 mW/cm^2 for sufficient time to permit substantial removal of irradiated photoresist by developer. In preferred embodiments, the photoresist is irradiated for between 1 and 2 minutes.

To enhance the contrast of light applied to the photoresist, contrast enhancement materials may be provided between the mask and the photoresist A contrast enhancement layer may comprise a molecule that is decomposed by light or transiently bleached by light. Transient bleaching of materials allows greater penetration where light is applied, thereby enhancing contrast. Poor contrast due to standing waves and reflective notching may be reduced by applying

an anti-reflective coating, for example, ARC® coating manufactured by Brewer Science Inc., Rolla, Mo. Alternatively, contrast enhancement may be provided by way of a cladded fiber optic bundle. The use of contrast enhancement materials is well known in the art.

As alternatives to the use of masks, other methods may be used to illuminate selected regions of photoresist. For example, the substrate may be translated under a modulated laser or diode light source (see Feyrer et al., U.S. Pat. No. 4,719,615). In alternative embodiments, a laser galvanometric scanner may be utilized. In other embodiments, the irradiation of the photoresist may take place on or in contact with a fiber optic light source, or a liquid crystal. By appropriately modulating liquid crystals, light may be selectively controlled so as to permit light to contact selected regions of the photoresist. Such a liquid crystal is also referred to as a "programmable mask," or an integrated circuit spatial light modulator (ICSLM), manufactured by Displaytech (Boulder, Colo.). Alternatively, irradiation may take place on the end of a series of optical fibers to which light is selectively applied. In some embodiments, light will be directed to extremely small regions, being limited by diffraction to a size directly proportional to the wavelength of light. In order to mask illumination to regions smaller than a wavelength of light, more elaborate techniques may be utilized. For example, light may be directed at the photoresist by way of molecular microcrystals on the tip of, for example, micropipettes (see Lieberman et al., *Science* 247:59, 1990). Other means of controlling the location of light exposure will be apparent to those of skill in the art.

After the irradiating step is completed, the photoresist is contacted with developer. This results in the selective, substantial removal of photoresist, and underlying porous coating, from irradiated (positive photoresists) or non-irradiated (negative photoresists) regions, leaving only photoresist and porous coating in discrete regions (see FIG. **1**C, illustrating the process for a positive photoresist). The developer is selected based upon the type of photoresist. For photoresists comprising a base soluble (e.g., phenolic polymer) component, the developer preferably has an alkaline pH, more preferably 9 to 12 pH units, and most preferably about 11 pH units. The developer may also contain various buffers and surfactants. For example, the developer may be a six-fold water dilution of AZ® 351 Developer, manufactured by Hoechst Celanese™, Somerville, N.J. Contact with developer may be by any suitable method, including immersion, although other methods of applying the developer exist including, for example, spraying, puddling and streaming. The rate of photoresist dissolution can be increased by increasing the pH or increasing the temperature, limited mainly by solubility considerations of remaining photoresist. In a preferred embodiment, irradiated photoresist is contacted with developer at a temperature from 20° C. to 30° C., and most preferably at a temperature from 23° C. to 27° C., for sufficient time to effect substantial removal of desired regions of the irradiated photoresist and underlying porous coating. Typically, both are completely removed after about 60 to 120 seconds.

In the absence of photoresist, the use of an alkaline developer as described herein results in no detectable dissolution of the porous coating. It was unexpectedly found, within the context of the present invention, that dissolution of irradiated photoresist results in the dissolution of the underlying porous coating. Although the actual mechanism is uncertain, it is known that the phenomenon is mitigated and even abolished by subjecting the porous coating to temperatures normally associated with high temperature curing. Presumably, the formation of oxane bonds during high temperature curing strengthens the porous coating against the putative forces that develop during the photopatterning process. Accordingly, it is necessary to avoid prolonged exposure to temperatures greater than about 100° C. until after the porous coating is patterned.

For photoresists not comprising a phenolic polymer, other developers (e.g., etchants) may be used to arrive at a patterned porous coating disclosed herein (suitable photoresists are reviewed extensively in *Desk Reference of Functional Polymers: Synthesis and Applications*, edited by Reza Arshady, (1997), American Chemical Society, Washington, D.C., incorporated herein by reference for all purposes). Suitable etchants for use in combination with alternative photoresists will be familiar to those skilled in the art and include, for example, reactive ion etchants, strong acids, strong bases, peroxide solutions, and mixtures thereof.

After treatment with developer, remaining photoresist is removed by contact with a stripping solution. The stripping solution is generally an organic solvent that selectively dissolves the photoresist, leaving only the patterned porous coating. In embodiments employing a phenolic polymer, the stripping solution may be, for example, a ketone, alcohol, amide, methanol, ethanol, isopropanol, 2-ethoxyethyl acetate, 1-methoxy-2-propyl acetate, or any of a wide number of organic solvents well known in the art. In a preferred embodiment, the stripping solution is acetone.

E. Application of Fortifying Solution

With or without photopatterning, a porous coating may, but need not, be treated with a fortifying solution comprising a polymeric binder to further anchor the elements of the porous coating without substantially filling the pore volume. One such binder is tetraethoxysilane. Other fortifying solutions are possible and include, for example, polymeric binders used to form the polymer-particle composites described above. In some embodiments, the fortifying solution is a 150-fold ethanol dilution of an aged solution comprising 21.7 ml of tetraethoxysilane in 6.3 ml H_2O and 0.7 ml 1N nitric acid. Such a solution may be applied to a porous coating as described above. Following evaporation of the solvent (e.g., at room temperature), a fortifying layer is left on the porous coating. After evaporation, the article is preferably cured at a higher temperature, as described below.

F. Curing

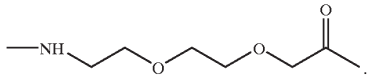
A porous coating may, but need not, undergo high temperature curing to increase the number of oxane bonds. The optimal degree of oxane bonding necessary for sufficient curing will depend on numerous factors including the desired application, primary particle size, and final film thickness. In particular, small particles require less oxane bonding than large particles due to the increased strength small particles confer to a coating (i.e., secondary to greater numbers of particle-to-particle contacts per unit volume). If a porous coating is used as a substrate to attach an array of compounds, further curing may be performed so that the degree of oxane bonding will be in a relatively high range to confer increased strength on the coating.

Using either the methods described above or empirical observations, the optimal degree of oxane bonding necessary for sufficient curing for a particular application and set of conditions may be readily identified by one of ordinary skill in the art. Curing is typically achieved by heating to a temperature of about 90° C. to 250° C. for a period of time sufficient to establish an extensive network of oxane bonds. For example, a porous coating may be cured at 110–120° C. for 15 minutes.

G. Attachment of Compounds

As noted above, a porous coating preferably has at least one compound attached thereto. Such compounds may be optional. Certain coated articles may comprise a substrate having a continuous porous coating thereon of substantially uniform thickness, wherein the porous coating comprises a gelled network of metal oxide particles and polymers of hydrolyzed metal alkoxide, wherein the porosity of the coating ranges from 0.15 to 0.99. Other coated articles may comprise a substrate having at least five separate distinct porous coatings per square centimeter, wherein each coating is continuous and has a substantially uniform thickness and comprises a continuous gelled network of particles. In general, however, coated articles having attached compounds are preferred. For articles containing only one porous coating, preferably at least two compounds are attached. For articles comprising multiple porous coatings (i.e., patterned porous coatings), one or more compounds may be attached to each porous coating.

To facilitate attachment of a compound, linkers may be used. A linker may serve a variety of functions, including spacing attached compounds from the surface, facilitating receptor recognition of attached ligands, or supplying a labile linkage that allows ligands to be detached from the surface. A spacer is a small molecule that serves to separate the synthesized compound from the surface Spacers may be used alone, or incorporated into linkers. Preferred spacers for incorporation into a linker include:

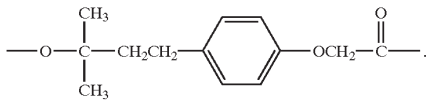


Linkers are preferably of sufficient length to allow an attached compound to interact with any desired reagents. Preferably, at least one linker at least 5 atoms long, is used, to permit free interaction between ligand and receptor, and multiple spacer molecules may be used to increase the length of a linker, if desired. Linkers may comprise, for example, aryl containing molecules, ethylene glycol oligomers containing 2–10 monomer units, diamines, diacids, amino acids, silane layers, or any of a wide variety of polymers such as polytetrafluoroethylene, polyvinylidenedifluoride, polystyrene, polycarbonate, polyethylene, polypropylene, nylon, polyvinyl alcohol, polyacrylamide, or combinations thereof. Other linker materials will be readily apparent to those of skill in the art.

In a preferred embodiment, linkers are organoalkoxysilanes containing one or more reactive groups. Reactive groups include, for example, amino (e.g., APES), hydroxy (e.g., HAPES), epoxy, carboxyl, sulfhydryl or halogen groups. Reactive groups are preferably on the distal or terminal end of the linker molecule opposite the surface. In preferred embodiments, the organoalkoxysilanes are 3-aminopropyltriethoxysilane (i.e., APES), bearing an amino group, and/or bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane (i.e., HAPES), bearing two hydroxyl groups.

Within other embodiments, a linker may be selected for hydrophilic or hydrophobic properties, to improve presentation of ligands to certain receptors. For example, in the case of a hydrophilic receptor, a hydrophilic linker is preferred so as to permit the receptor to more closely approach the synthesized ligand.

Alternatively, or in addition, a linker may be selected to permit removal of an attached compound. Such a linker may be, for example, photocleavable, acid labile, base-labile or cleavable by an enzyme. The use of a photocleavable linker permits removal of ligands by irradiation with light at a wavelength that may be chosen to be distinct from wavelengths used to perform other process steps (including, for example, photopatterning of the porous coating and ligand-array synthesis). Within a photocleavable linker, the cleavable portion is preferably located at an intermediate position between the distal end of the linker and the end attached to the substrate. More preferably, the cleavable portion is located at the distal end such that photocleavage leaves no remnants of the linker on the detached compound. An acid- or base-labile linker comprises a labile moiety that permits the removal of ligand upon exposure to acid or base. An acid or base may be, for example, vapor-phase trifluoroacetic acid (TFA) or NH_3, respectively. Acid-, base-, and photolabile linker molecules are known in the art, and are commercially available (see *The Combinatorial Chemistry Catalog*, Nova Biochem, Inc., 1998). One suitable acid labile linker has the formula:



Both photocleavage and vapor-phase cleavage of ligand-arrays allow separated ligands to remain co-localized with their site of attachment and/or synthesis. Ligand separation from the support is essential for the formation of many ligand-receptor pairs. Co-localization is particularly advantageous when an in situ assay is used to determine ligand-receptor binding. In such an assay, determining the location of binding also determines the identity or reagent history of the bound ligand. It is particularly preferable to screen arrays of drug candidates using in situ assays.

A linker may also, or alternatively, comprise a recognition sequence for cleavage by an enzyme, preferably at an intermediate position. Such a sequence enables removal of ligands by contact with enzymes. An enzyme-cleavable group may be chosen so as to be substantially cleavable with a protease, non-specific nuclease, specific nuclease or enzyme secreted by a cell. Preferably, the enzyme-cleavable moiety connects the linker with the ligand so as to enable the removal of ligand upon contact with a living cell. Most preferably, the cell will secrete an enzyme that detaches the ligand from the array which subsequently diffuses into the cell and affects some internal biologic process. For example, arrays of nucleobase polymers attached via protease-sensitive linkages may be used to conduct arrays of antisense experiments on cells growing in direct contact with the surface of the array. Ligand separation from the support is essential for transmigration of the ligand through the cell membrane. Cell-induced cleavage of the nucleobase polymer also allows the separated ligands to remain co-localized with their site of attachment and/or synthesis. Co-localization is particularly advantageous when a phenotypic cellular assay is used to determine modulation of gene expression by the nucleobase polymer. In such an assay, determining the location of the phenotypic change determines the sequence of the nucleobase polymer affecting the change, as well as the base sequence of its intracellular target.

A linker may be covalently attached or adsorbed to the surface (via C—C, C—N, C—O, C—S, Si- or other chemical bonds) according to methods well known in the art (see *Methods in Enzymology*, vol. XLIV, edited by Klaus Mosbach, (1976), Academic Press N.Y.). For example, link-

ers with hydroxy groups may be attached to a surface with a 2% solution of HAPES in 95:5 ethanol:H_2O for 10 minutes, followed by rinsing with ethanol and curing at 120° C. for 15 minutes. Linkers with amino groups are attached similarly except that APES is substituted for HAPES. Organoalkoxysilanes may generally be attached to a surface via siloxane bonds.

Alternatively, linkers may be incorporated into the gelled network of the porous coating by copolymerization, such that the linkers are present throughout the thickness of the porous coating. For example, in one embodiment, an amino-modified coating solution may be made by mixing 50.0 ml of 5 weight percent silica particles dispersed in 95% ethanol/5% H_2O (500 Å primary particle size), 0.435 ml of 6 mM HNO_3, 0.100 ml of tetraethyoxysilane (180 μmole/g of silica particles), and 0.035 ml of APES (60 μmole/g of silica particles). The coating solution may be mixed at room temperature for two days, and yields a porous coating that directly couples to amino-reactive reagents. It is, however, preferred to attach linker molecules after forming the porous coating.

Compounds may be attached to a porous coating within separate full-thickness volumes, with or without linkers, using well known techniques. Attached compounds may have molecular weights less than about 10^1 gram/mole, 10^2 gram/mole, 10^3 gram/mole, 10^4 gram/mole, 10^5 gram/mole, 10^6 gram/mole, or 10^7 gram/mole. Such compounds may be of any type including, for example, nucleobase polymers (see glossary), pharmacologic agents, drug analogues, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either α-, β-, or ω-amino acids, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polysiloxanes, polyimides, polyacetates or other ligands.

In preferred embodiments, a coated article comprises an array of ligand groups attached to the porous coating within separate full-thickness volumes. Arrays of ligands may be attached to the porous coating using any placement method that is compatible with the synthesis of compounds on a porous three dimensional object including, for example, ink-jet technology (see Brennan, U.S. Pat. No. 5,474,796). Most preferably, however, ligand arrays are formed on the porous support using methods and compositions described more fully in co-pending Application Ser. No. 09/326,479 entitled, "Methods and Compositions For Performing an Array of Chemical Reactions on a Support Surface" and U.S. Pat. No. 6,569,598, entitled "Solvent-Resistant Photosensitive Compositions." Synthesis methods employing photoremovable groups are not generally compatible with the porous coatings described herein, as a result of incomplete photodeprotection, and such techniques should generally be avoided.

Within certain embodiments, compounds may be synthesized on the surface of the porous coating by sequential coupling of chemical precursors using, for example, methods collectively known in the art as "solid-phase synthesis." An important aspect of the present invention is the discovery that the porous coatings provide excellent supports for performing solid-phase chemical synthesis of ligands and detecting bound ligands with labeled macromolecular receptors, using a variety of protocols and reagents. In contrast to supports that require swelling and solvation for efficient mass transfer of reagents, the rigid porous network of the present invention is permanently open and resides on the surface of the substrate. Reactive groups on the surface are therefore substantially accessible by any reagent by directly contacting the support. Accordingly, the flow-through apparatus required to apply reagents to the parallel and highly elongated sub-surface pores of the acid-etched porous silicon and electrochemically manufactured metal oxide membrane of the prior art is not necessary (see Beattie et al., *Clin. Chem.* 41:700, 1995 and Van Damme and Kreuwel, WO99/02266). Ligands may be synthetically established on the surface by any number of solid-phase synthesis methods familiar to those skilled in the art, including but not limited to, solid-phase nucleic acid synthesis (e.g., phosphoramidite or H-phosphonate methods), solid-phase peptide synthesis (e.g., the "Merrifield Method", see Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963 and subsequent improvements thereto), solid-phase peptide nucleic acid synthesis (see Egholm et al., *J. Am. Chem. Soc.* 114:1895, 1992), solid-phase nucleobase polymer synthesis (see Summerton and Weller, U.S. Pat. No. 5,185,444; Shah et al., U.S. Pat. No. 5,698,685; Stirchak and Summerton, *J. Org. Chem.* 52:4202, 1987; Lebreton et al., *Synlett.* 137, 1994; Vasseur et al., *J. Am. Chem. Soc.* 114:4006, 1992; Jones et al., *J. Org. Chem.* 58:2983, 1993; Huie and Trainor, U.S. Pat. No. 5,470,967 and Swaminathan et al., U.S. Pat. No. 5,817,781), and solid-phase small-molecule synthesis (see *The Combinatorial Chemistry Catalog*, Nova Biochem, Inc., 1998).

Briefly, solid-phase synthesis may be achieved by one or more cycles through a series of four steps: (1) application of photoresist; (2) irradiation of photoresist and removal of a portion of the photoresist; (3) contact of exposed molecules with a reagent; and (4) removal (or stripping) of remaining photoresist. Each of these steps is described in greater detail below, and the series may be performed as many times as needed to generate the attached compounds of interest.

In general, chemical reactions performed on a surface may be characterized by the reagents used. For example, a reaction defined by the addition of a reagent R_1 is designated by the notation $[R_1]$, where the square brackets indicate the process of contacting the support with a reagent. The order of reagents contacted with a region define its reagent history. Accordingly, after a first cycle, exposed regions of a surface comprise ligands with the following reagent history:

S-L-[R_1],

wherein S indicates the surface and L indicates a linker, while remaining regions of the surface comprise ligands with the following null reagent history:

S-L-[Ø]

After a second cycle of photoresist application, irradiation, exposure to developer and contact with a second reagent R_2 (which may or may not be the same as R_1), different regions of the support may comprise ligands with one or more of the following reagent histories:

S-L-[R_1]-[R_2]

S-L-[R_2]

S-L-[R_1]

S-L-[Ø]

The above process is repeated until a plurality of ligands are attached to the substrate, each in discrete known regions and each with a known reagent history. In preferred embodiments, the reagent history will determine the predominant ligand composition at a predefined region. Thus, by controlling the regions of the support masked by photoresist and the reagent history of each region, the location and composition of each ligand will be known.

a. Application of Photoresist

To begin synthesis of attached compounds, the porous coating (and linkers) are covered by a layer of photoresist. Any suitable photoresist may be used for this purpose, provided that (1) the photoresist provides a barrier layer (2) irradiation of the photoresist results in differential solubility of the photoresist in irradiated regions, relative to non-irradiated regions; (3) such irradiation can be performed with light of a wavelength that does not result in substantial photodegradation of surface-attached molecules, (4) the photochemical reaction undergone by the photoresist is substantially inert with respect to surface-attached molecules in contact with the photoresist; (5) a suitable developer, if needed, is substantially unreactive with the surface and attached molecules and (6) the photoresist is substantially removable by stripping solutions that are substantially inert with respect to the underlying molecules and substrate.

The barrier layer provided by the photoresist should be sufficient to prevent detectable reaction of a reagent with underlying molecules under conditions that permit such a detectable reaction with such molecules that are not covered by the photoresist. The barrier layer should be at least 0.1 microns thick and should form a continuous coating. Preferably, the barrier layer is substantially impermeable to organic solvents to be used in the synthesis reactions. This property may be assessed by generating a layer, contacting one side of the layer with an organic solvent of interest, and determining whether the solvent passes through the layer under conditions that are to be used in the assay. Diffusion of solvent into the layer may be detected by testing for evidence of layer swelling. In general, a barrier layer is substantially impermeable to a solvent if its thickness increases (i.e., swells) by less than 50% at equilibrium, as determined by interferometry or profilometry. Such solvent-impermeability is desirable but is not an absolute requirement.

As described above in the context of photopatterning, irradiation of a photoresist barrier layer with a specific wavelength of light permits the selective, substantial removal of photoresist from irradiated (positive photoresists) or non-irradiated (negative photoresists) regions. In general, for a positive photoresist, a differential solubility of at least 20-fold is sufficient to produce a useful photoresist system. For example, irradiation and exposure to developer resulting in removal of at least 2 microns of a photoresist in irradiated regions, should result in the removal of no more than 0.1 microns in non-irradiated regions, as determined by profilometry.

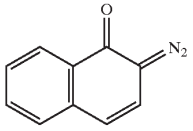
Within the methods provided herein, such irradiation should not result in detectable alteration of the underlying molecules. Thus, a suitable photoresist should be reactive to light of a wavelength that does not result in detectable degradation of the underlying molecules. For most applications, the light should have a wavelength greater than that which causes direct photodegradation of molecules (i.e., >260 nm, preferably >300 nm). Those of ordinary skill in the art will be readily able to determine specific wavelengths that are suitable for use in the synthesis of a desired compound. Further, the chemistry that takes place within the photoresist layer upon irradiation should be substantially inert with respect to the underlying molecules. Irradiation of the photoresist should not result in reactive compounds that may react with the compounds to be synthesized. Similarly, any developer employed, and stripping agents for removal of photoresist, should not modify the underlying molecules. In other words, developers and stripping solutions should result in substantial removal of the photoresist without degrading the surface or attached molecules. In general, the process agents comprising irradiation, photochemical reactions in the photoresist, developers, and strippers should produce less than 50%, and more preferably less than 10% degradation of compounds each time they are used. Degradation may be measured by assessing reaction yields in the presence and absence of the above process agents (see Glossary phrase "substantial removal").

Suitable photoresists for use in solid phase synthesis may be identified by considering the properties of the photoinactive and photoactive components of the photoresist separately. The photoinactive component of a photoresist determines the majority of the bulk properties of a photoresist including solvent-resistance (i.e., insolubility in a particular solvent), and is typically a polymer. Suitable candidates for the photoinactive component may generally be selected from those polymers whose solvent-resistance includes the reagent solvent to be used in a particular synthetic reaction. A vast array of polymers and their solubility profiles in various solvents have been described in the art (reviewed by Fuchs in: Polymer Handbook, 2nd edition, Wiley-Interscience, New York, edited by Brandrup and Immergut (1975), p. 379). Depending on the desired solubility profile of the photoresist, candidates for the photoinactive component may be selected from poly(dienes), poly(acetylenes), poly(alkenes), poly(acrylates), poly(acrylic acids), poly(methacrylics), poly(disubstituted esters), poly(acrylamides), poly(methacrylamides), poly(vinyl ethers), poly(vinyl alcohols), poly(acetals), poly(vinyl ketones), poly(vinyl halides), poly(vinyl nitrites), poly(vinyl esters), poly(styrenes), poly(phenylenes), poly(oxides), poly(carbonates), poly(esters), poly(anhydrides), poly(urethanes), poly(sulfonates), poly(siloxanes), poly(sulfides), poly(sulfones), poly(amides), poly(hydrazides), poly(ureas), poly(carbodiimides), poly(phosphazenes), poly(silanes), poly(silazanes), poly(benzoxazoles), poly(oxadiazoles), poly(oxadiazolidines), poly(dithiazoles), poly(benzothiazoles), poly(pyromellitimides), poly(quinoxalines), poly(benzimidazoles), poly(piperazines), poly(anhydrides), poly(formaldehydes), poly(phosphonates), poly(phosphates) and poly(thiophosphonates). Preferred polymers are those with narrow solubility profiles and include, for example, polyethylene (low density), polypropylene, poly(di-n-butyl itaconate), polyacrylamide, poly(vinyl alcohol), poly(allyl alcohol), poly(chlorotrifluoroethylene), poly(2,5-dimethoxy-1,4-phenyleneethylene), poly(oxy-1,4-phenyleneoxyisophthaloyl), poly(1-butene-co-sulfur dioxide), poly(imino(1-oxotrimethylene)), poly(1,3,4-oxadiazoles), poly(dibenzoxazole), poly(dithiazoles), poly(pyromellitimides), poly(benzimidazoles), poly(dibenzimidazoles), poly(oxypropylidene), polyamic acids and polyimides. Particularly preferable are the aromatic polyamides or "aramids" which have a very narrow solubility spectrum, being soluble mainly in n-alkyl amide solvents, as described by Preston in: *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 3, 3rd edition, edited by Grayson and Eckroth (1978), Wiley-Interscience, New York, p. 213). In some embodiments, the photoinactive component may be a polymeric blend, wherein the blend confers enhanced solvent resistance as in, for example, the blend of certain amorphous polyamides and polyesters reported by Clagett et al. U.S. Pat. No. 5,346,967.

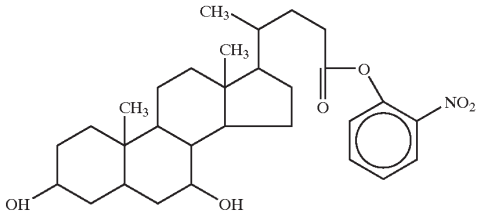
The photoactive component results in a change in the bulk properties of the photoresist subsequent to irradiation such that either irradiated or non-irradiated portions are removed selectively. Typically, the photoactive component changes the solubility of the photoresist in a particular liquid developer. The photoactive component may comprise a single molecule or may comprise two or more molecules in a "photoreactive system." The photoactive component may be an integral part of the photoinactive polymer through covalent attachment, or may exist as a miscible blend with the photoinactive polymer.

Suitable photoactive candidates may be selected from those photoreactive molecules that effect a change in the solubility profile of the photoinactive polymer while not adversely affecting the molecules attached to the array surface. Photoactive components with these properties may be selected by identifying those photoreactive molecules that undergo substantially intramolecular photoreactions, or photoreactions that are highly specific for a class of molecules not attached to the array surface. The photoactive component is further selected based on the wavelength of light necessary to affect a substantial photoreaction. Preferably, the photoactive component reacts to radiation in the ultraviolet (UV) or visible portion of the electromagnetic spectrum. More preferably, the photoactive component will be reactive to radiation in the near UV or visible portion of the spectrum having a reactivity to light with a wavelength greater than about 250 nm, 300 nm, 350 mm or 400 nm. Numerous photoactive components which fulfill these criteria have been described, and will be familiar to those of skill in the art.

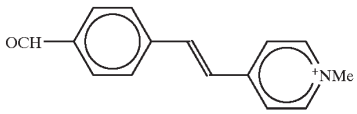
A preferred class of photoactive components comprises molecules that inhibit the solubility of the polymeric component in a miscible blend with the polymer (see *Desk Reference of Functional Polymers: Synthesis and Applications*, edited by Reza Arshady, (1997), American Chemical Society, Washington, D.C., Chapters 2.1, 2.2, and 2.3). Such dissolution inhibitors have been used to produce both positive and negative photoresists. Preferable dissolution inhibitors are those photoactive molecules that undergo a substantially intramolecular photoreaction. These include, for example, diazoquinones:



A very large variety of diazoquinone derivatives has been described in the patent literature and will be familiar to those skilled in the art (see DeForest, Photoresist Materials and Processes, McGraw-Hill (1975)). For example, diazoquinones have been successfully used as the miscible photoactive component in polyimide-based photoresists (see Yukawa and Kohtoh, U.S. Pat. No. 5,288,588 and Oba et al., U.S. Pat. No. 5,348,835). Other preferable dissolution inhibitors that undergo intramolecular photoreactions include o-nitrobenzyl cholates (see Reichmanis et al., *J. Vac. Sci. Technol.* 19:1338, 1980):

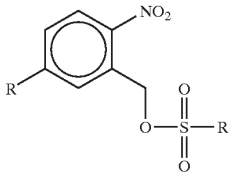


Alternatively, a negative photoresist may be formulated by combining a polymer with a photoactive component that is a cross-linking agent. Preferred cross-linking agents are those that do not react with the molecules attached to the array surface, such as those derived from stilbazolium (SBQ) substituted polymers (see U.S. Pat. Nos. 5,445,916 and 4,891,300):



A unique property of SBQ substituted polymers is that non-covalent dimers of SBQ form in the solid-state. Because SBQ units are non-covalently paired before irradiation, photoreactive species are paired and do not participate with the underlying material on the array surface.

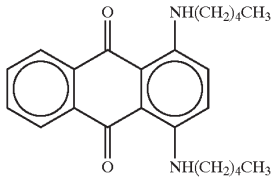
Photoresists may also be formulated by masking a solubilizing functionality on the polymer. For example, the photoresist may be a chemically-amplified photoresist produced by combining a photoacid generator with a polymeric component derivatized at solubilizing functionalities with acid-labile groups such as tert-butoxycarbonyl (t-Boc), benzhydryloxycarbonyl (Bhoc), trimethylsilyl, t-butyl, phenoxyethyl, or tetrahydropyranyl. Preferable photoacid generators are those that do not react with the molecules attached to the array surface. For example, a preferred class of suitable photoacid generators are those which undergo substantially intramolecular reactions such as, for example, o-nitrobenzyl esters of sulfonic acids as follows:



In other embodiments, the photoacid generator initiates acid-catalyzed depolymerization of the polymeric component resulting in the production of volatile components that obviate the need for wet developer processing. A preferred acid-catalyzed depolymerization reaction for polyphthalaldehyde is described by Willson et al., *J. Electrochem. Soc.: Solid-State Science and Technology* 133(1): 181, 1986.

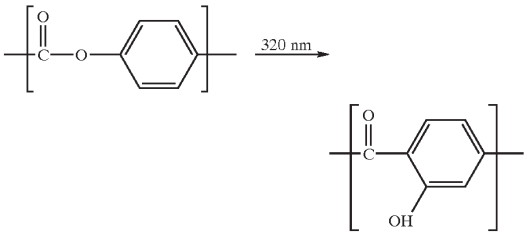
In other embodiments, photoresists that do not require wet development may be used. Such photoresists include dye-in-polymer composites, wherein the dye assists in absorbing radiant laser energy of a particular wavelength resulting in photoablation of the photoresist by concentrated laser irra-

diation (see Law, *J. Appl. Phys.* 54(9):4799, 1983 and Law and Vincett, *Appl. Phys. Lett.* 39(9):718, 1981). The wavelength is one not typically absorbed by organic molecules, leaving attached organic molecules unaffected be the incident laser irradiation. Preferred dyes include, for example, oil nile blue (λ_{max}=644 nm):



In other embodiments, the photoactive component may itself be used to mask solubilizing functionalities on the polymeric component. Preferred photoactive components for masking solubilizing functionalities include o-nitrobenzyl and N-alkyl-o-nitroanilide groups (see review by Pillai, Synthesis 1980 (1980) p. 1). Several photoresists have been described that incorporate masking groups based on o-nitro chemistries (see Kubota et al., *J. Appl. Polymer Sci.: Polymer Chem. Ed.* 33:1763, 1987). Such compounds are known to undergo predominantly intramolecular photoreactions. Particularly preferable o-nitro-based masking groups are those described by Fodor et al., U.S. Pat. No. 5,424,186.

In still other embodiments the photoactive component is attached to the polymeric component and undergoes a light-induced rearrangement to produce a solubilizing functionality. Preferred rearranging groups include diazoquinones, which have been successfully used as the photoactive adduct in polyimide-based photoresists (see Khanna, U.S. Pat. No. 5,037,720). Other preferred rearranging groups include those comprising phenyl esters, phenyl carbonates, or phenyl ethers. Such groups undergo an intramolecular photo-Fries rearrangement yielding a solubilizing hydroxyl group, as in the reaction shown below:

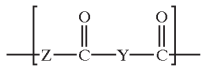


Other photoresists may be formulated by providing photolabile linkages within the polymeric component that result in a reduction in the molecular weight of the polymer and a concomitant increase in solubility. Preferred photolabile linkages include those found in polysilanes and polysulfones (see *Desk Reference of Functional Polymers: Synthesis and Applications*, edited by Reza Arshady, (1997), American Chemical Society, Washington, D.C., p. 297–300). Disilane and sulfone linkage may be incorporated into other photoinactive polymers as well. More preferred photolabile linkages include those based on o-nitrobenzyl and N-alkyl-o-nitroanilide chemistries (see Petropoulos, *J. Appl. Polymer Sci.: Polymer Chem. Ed.* 15:1637, 1977; lizawa et al., *J. Polymer Sci.: Part A: Polymer Chem.* 29:1875, 1991; and MacDonald and Willson, in: *Polymeric Materials for Electronic Applications*, ACS Symp Ser. 184, American Chemical Society, Washington, D.C., edited by MacDonald et al., (1982), p. 73).

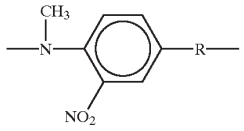
It will be apparent that there are many different photoresist compositions that are suitable for use within the methods provided herein. Based on the teachings of the present specification, those of ordinary skill in the art will be readily able to optimize a photoresist system for a particular application using only routine analyses.

In preferred embodiments, the photoresist is as described in U.S. Pat. No. 6,569,598 entitled "Solvent-Resistant Photosensitive Compositions." Such a photoresist generally comprises a polyamide derivative formed by the condensation of (1) a diamine mixture comprising a N-alkyl-2-nitro diamine and at least one of 1,4-phenylenediamine or 1,3-phenylenediamine and (2) a diacid chloride mixture comprising isophthaloyl chloride. Preferred N-alkyl-2-nitro diamines include N^1-methyl-2-nitro-p-phenylenediamine and 3,3'-dinitro-4,4'-di-N-methylaminodiphenyl ether. Preferred mole ratios of the diacid mixture to the diamine mixture range from 0.980 to 1.020.

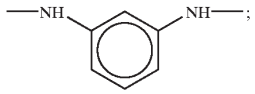
One such photoresist comprises a polyamide derivative having a repeating unit represented by the following general formula:


(1)

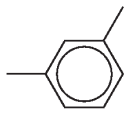
where Z is 20 to 50 mole percent, and more preferably 20 to 35 mole percent, a structure comprising:



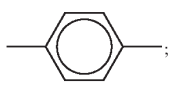
with the balance comprising



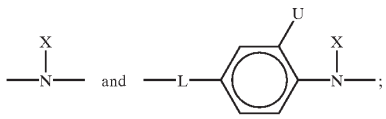
and Y is 10 to 100 mole percent a structure comprising:



with the balance comprising



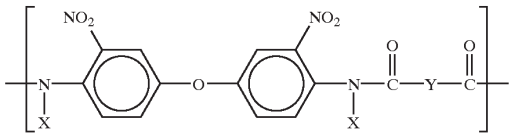
and R is a divalent organic group without particular restrictions. In some embodiments R may be selected from the group consisting of:



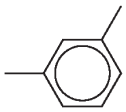
where X is H or CH_3; L is direct link, O, CH_2, $N(CH_3)$, $C(CH_3)_2$, $C(CF_3)_2$, SO_2, CO, CONH, $O(C_6H_4)_2$, S, $C(C_6H_5)_2$ or $C(CF_3)(C_6H_5)$; and U is H, NO_2 or CH_3. In preferred embodiments R is NH.

In a second embodiment, the photoresist comprises a polyamide derivative having a repeating unit represented by the following general formula:

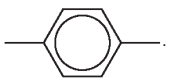
(2)



where X is 10 to 100 mole percent CH_3 with the balance H; and Y is 0 to 50 mole percent a structure comprising:

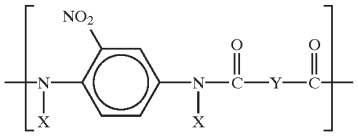


with the balance comprising

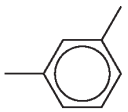


Within a third embodiment, the photoresist comprises a polyamide derivative having a repeating unit represented by the following general formula:

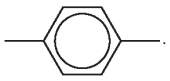
(3)



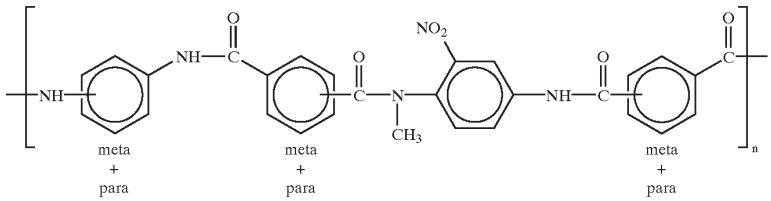
where X is 10 to 50 mole percent CH_3, and more preferably 10 to 20 mole percent CH_3, with the balance H; and Y is 20 to 100 mole percent a structure comprising:



with the balance comprising

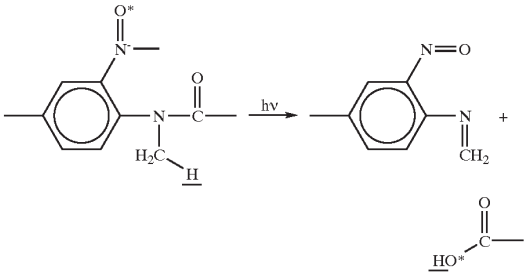


Certain preferred polyamide photoresists may be represented by the following formula:



In a preferred embodiment, the polyamide is formed by condensing N^1-methyl-2-nitro-p-phenylenediamine, 1,4-phenylenediamine, 1,3-phenylenediamine, isophthaloyl chloride, and terephthaloyl chloride, using mole percentages of 17.67, 0.0, 32.82, 24.75, and 24.75, respectively. After polymerization, polymer ends may be capped by further condensing the polymer with benzoyl chloride. Irradiated regions of the photoresist undergo cleavage at the N-methylated amide bond forming free carboxyl groups and reducing the molecular weight of the polymer. The irradiated regions may be solubilized by a developer comprising a mixture of ethanolamine and cyclohexanone. In preferred embodiments the developer is 10–15% ethanolamine in cyclohexanone. Unirradiated photoresist may be removed by amide-based strippers including, for example, 1-methyl-2-pyrrolidinone and dimethylformamide.

The above polyamide compositions provide dry films that are resistant to numerous solvents. Irradiation of these films with 365 nm light results in intramolecular photo-oxidation as follows:



This reaction is known to be substantially intramolecular (for a review, see Pillai, Synthesis 1980 (1980) p. 1). As such, irradiation does not result in side-reactions with surface-attached groups in contact with the film. Irradiated regions may be selectively solubilized by non-aqueous developers. Without wishing to be bound by any particular theory, the photopatterning mechanism is believed to be a consequence of both polymer chain cleavage, and the appearance of acidic carboxyl groups.

A photoresist may be applied using standard techniques, as described above, including application with a pipette on an incline, dip-coating, spin-coating and microdispensing. Optimal spreading of liquid photoresist requires that the surface tension of the substrate be compatible with wetting by the photoresist solvent. For example, solvents with a surface tension below the critical surface tension of a substrate will wet the substrate surface (i.e., show a contact angle of zero). It will be apparent to those of ordinary skill in the art that contact of certain substrates with solutions at a particular pH may be necessary to create the appropriate surface tension by altering the density and/or composition of static charge on the surface. In one embodiment the substrate is briefly contacted with a basic aqueous solution (e.g., AZ® 351 developer) prior to the application of photoresist. Other methods of altering the surface tension to provide wetting by a liquid photoresist will be familiar to those of ordinary skill in the art. All operations in the process of applying, irradiating and developing the photoresist should be carried out in a room lit primarily or entirely by light of a wavelength outside of the light range which will react with the photoresist.

After a photoresist solution is applied, the photoresist layer may be generated by heating. For example, a substrate may be baked at about 85° C. to 90° C. for a few minutes until substantially all the solvent has evaporated. In preferred embodiments, photoresist coating is 0.2 μm to 4.0 μm thick. Following this soft-bake, a substrate may be further baked for several minutes at 110° C. to 135° C. to ensure complete solvent removal. Incomplete solvent removal may lead to a coating that loses integrity upon contact with various solvents.

Following application, the photoresist should be continuous and cover any underlying molecules. More specifically, the underlying molecules should reside under a layer of photoresist from 0.1 to 20 microns thick, and more preferably 1 to 3 microns thick. In embodiments that employ molecules attached to raised elements such as, for example, a plurality of porous coatings, the photoresist should also cover these elements as well. Depending on the thickness of the photoresist the surface of the photoresist will be flat or will follow the surface contour of the substrate and raised and/or depressed regions or elements. In general, the surface contour of the photoresist will be at least 0.1 microns higher than the surface contour of the attached underlying molecules.

b. Irradiation

The photoresist layer is then selectively irradiated (i.e., a portion of the photoresist is irradiated with a wavelength that alters the solubility of the irradiated region). Such selective irradiation may be achieved using one or more masks and photolithographic techniques as described above. In certain embodiments, the irradiation of the photoresist may itself result in substantial removal of the irradiated photoresist. Within other embodiments, the irradiated photoresist layer must be exposed to a developer to facilitate photoresist removal. The developer may be a solution that selectively solubilizes and removes irradiated or non-irradiated regions. In photoresist embodiments employing photoreactions that proceed by a non-crosslinking mechanism, developers may be identified by testing solvents and solvent mixtures that fall outside the solubility spectrum of the polymeric component. Often the photoactive component in such photoresists results in the production of a basic hydroxyl or carboxylic moiety and selective solubilization of irradiated portions can be achieved by the addition of an aqueous or organic base to the solvent or solvent mixture. Preferable organic bases include, for example, triethylamine, ethylamine, ethanolamine, triethanolamine, morpholine, piperidine, and diisopropylethylamine. Using these guidelines, selected solvent and base mixtures can be rapidly tested for developer activity in a panel format using several coated substrates irradiated in parallel through a test mask pattern. For photoresists based on photo-crosslinking, preferable developer solutions are most readily identified by testing solvents that are known to be within the solubility profile of the polymeric component.

Suitable developers comprise non aqueous mixtures of solvents containing ketone, amino, hydroxyl and/or amide moieties, such as N-methylpyrrolidone, dimethylacetamide or dimethylformamide. Representative mixtures which may be used to develop each of the embodiments represented by formulas (1), (2), and (3) are shown in Table I. Alternative developers may be gaseous compositions or irradiation.

TABLE I

Photopolymer Formula	Developer Solutions (volume %)
1	a. 15% ethanolamine, 85% cyclohexanone
	b. 15% ethanolamine, 35% acetone
2	a. 40% NMP, 60% ethanol
	b. 50% ethanolamine, 50% formamide
	c. 11% ethanolamime, 89% methanol
3	a. 10% triethanolamine, 90% acetone
	b. 25% DMF, 25% ethanolamine, 50% acetone

DMF is dimethylformamide

In general, a photoresist should be allowed to remain in contact with a developer solution until the photoresist coating has been substantially removed from irradiated regions of a positive photoresist (or non-irradiated regions of a negative photoresist). In preferred embodiments, this requires from 5 to 10 minutes of immersion. Regardless of the nature of the developer, exposure of the photoresist to developer results in a photoresist layer with one or more openings that expose the underlying molecules (or surface) in the irradiated region(s), for a positive photoresist.

After completion of exposure to developer, the photoresist layer may be rinsed with a suitable volatile solvent so as to remove residual developer and/or removed photoresist. One suitable rinse solvent is acetonitrile. A post-rinse heat treatment or bake may be employed to further increase the solvent-resistance of the film. In some embodiments, the film is heated at a temperature from about 90° C. to 135° C. for about one minute.

c. Contact with Reagent (s)

The regions from which photoresist has been removed are then contacted with at least one reagent. Preferably, the entire photoresist layer is contacted with the reagent, which reacts only with first molecules in exposed region(s). Liquid reagents may be applied to the support surface using several techniques including, but not limited to spraying, dipping, microdispensing or combinations thereof. Although reagents are preferably applied to the surface using solution-phase methods, it will be apparent to those skilled in the art that vapor-phase methods are also possible.

In preferred embodiments, liquid reagents may be delivered using a reactor system depicted in FIG. 1E. The elements of such a reactor system may be held together with a clamp. The reactor cavity may have any suitable volume (e.g., 300 μl, which is sufficient to encompasses a 1.25 cm×1.25 cm region of porous coating). In preferred embodiments, the reactor base and gasket are polytetrafluoroethylene, and the substrate is glass. The reactor system allows chemical reagents to be delivered over the porous coating either manually or automatically by connecting the inlet and outlet ports to either syringes or a reagent delivery machine, respectively.

The types of reagents that may be used to construct a history are without restriction. In preferred embodiments, the reagents are components of solid-phase synthesis methods that yield biopolymers or pharmacologic analogues. Reagents are preferably precursors of organic polymers such as polynucleotides, polypeptides, peptide nucleic acids, morpholino-based nucleobase polymers, peptide-based nucleic acid mimics (PENAMs) and nuclease resistant polynucleosides.

Biopolymer ligands may be synthetically established on the surface by solid-phase nucleic acid synthesis (e.g., phosphoramidite or H-phosphonate methods), solid-phase peptide synthesis (e.g., the "Merrifield Method", see Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963) or solid-phase peptide nucleic acid synthesis (see Egholm et al., *J. Am. Chem. Soc.* 114:1895, 1992). Agents with known or potential pharmacologic activity available by solid-phase synthesis include, for example, analogues of benzodiazepine, sulfonamide, hydantoin, miconazole, dihydropyridone, pyrazolone, pyrimidine, quinazoline, quinazolinone, oligocarbamates, peptoids, peptidyl phosphonates, and carboxyalkyldipeptides (see Gordon et al., *J. Medicinal Chem.* 37:1385, 1994 and *The Combinatorial Chemistry Catalog*, Nova Biochem, Inc., 1998). Other small-molecule syntheses are possible using organic reactions known to occur on the solid-phase. Illustrative examples of such reactions are shown in Table II.

TABLE II

Transformation	Reaction or Product	Transformation	Reaction or Product
Aromatic substitution	Heck reaction/olenification	Electrocyclic reactions	2 + 3
	Suzuki reaction		2 + 2
	Nucleophilic and Pd mediated		2 + 4
	Fischer indole synthesis		Pauson-Khand reaction
			Ring-closing metathesis
Condensations	Aldol reaction	Cleavage	Amination
	Mannich reaction		Cyclization
	Dihydropyridone		2 + cycloaddition
	Perhydrodiazepinedione		Hofmann elimination
	Pyrazolone		Ring closing metathesis
	Pyrimidine		Transesterification
	Quinazoline		Activation by acylation
	Quinazolinone		Organocuprate reaction
Radical reaction	Radical cyclization	Carbene	Arndt Eistert homologation
Michael addition		Halogenation	
Olefination	Aza Wittig	Organometallic	Grignard reaction
	Horner-Emmons/Wittig		Organolithium
Reductions	Imine to amine	Alkylations	N-alkylation
	Azide to amine		C-alkylation
	Nitro to amine		S-alkylation
	Amide to amine		O-alkylation
Amide formation	Carbamate	Oxidations	Alcohol to aldehyde
	Sulfonamide		Alkene to epoxide
	Urea		Sulfide to sulfoxide
			Sulfide to sulfone

from The Combinatorial Chemistry Catalog, Nova Biochem, Inc., 1998.

Reagents may also be components of solid-phase synthesis strategies that use enzymatic methods, such as the polymerase chain reaction (PCR), in vitro RNA synthesis using an RNA polymerase, and protein synthesis using an in vitro protein translation system (e.g., reticulocyte lysate systems). Alternatively, reagents may be components of methods that couple intact ligands to the surface (see *Methods in Enzymology*, vol. XLIV, edited by Klaus Mosbach, (1976), Academic Press N.Y.). Other reagents and solid-phase synthesis methods available for attaching ligands to the substrate will be apparent to those of ordinary skill in the art.

Depending on the reagent history, a predefined region may sustain a sequence of chemical reactions that include bond coupling, bond cleaving, bond rearranging, or any combination thereof. Such bond changes typically occur in both the reagent and the attached molecules, but in some cases may occur only in one or the other. Chemical reactions may make groups on the attached molecule reactive in subsequent chemical reactions, or may deactivate or block groups from subsequent chemical reactions. In many embodiments, the last reagent in the reagent history will remove protective groups from one or more of the attached molecules. In some embodiments, the reagent history may lead to regions with attached polymers such as, for example, peptides, DNA or PNA.

In some embodiments, a plurality of reagents are sequentially contacted with a given patterned photoresist layer. In

other embodiments, reagent histories are interspersed with reagents added without photoresist layers. Such reagents contribute to a plurality of ligands having reagent histories that have common sub-histories. For example, it may be desired to synthesize ligands with a reagent history of S-[R_1]-[R_2]-[R_3] at first regions and ligands with a reagent history of S-[R_4]-[R_2]-[R_3] at second regions. The process would begin by-establishing a photoresist layer and irradiating it in a first region. The photoresist is then contacted with developer, contacted with reagent R_1, and stripped. A second photoresist layer is established and irradiated in a second region. The photoresist is contacted with developer, contacted with reagent R_4, and stripped. First and second regions are then simultaneously contacted with reagent R_2 followed by reagent R_3 without photoresist layers, leaving the common sub-history [R_2]-[R_3] at both regions. The number of reagents in a common sub-history could cover a wide variety of values, but in preferred embodiments ranges from 2 to 100, 2 to 20, and most preferably 2 to 3.

In some embodiments, reagents added without photoresist layers may react differently in different regions depending on the effect of reagents added previously using photoresist layers. As an illustration, suppose it is desired to synthesize ligands with a history of S-X-[R_1]-[R_2] at first regions, and ligands with a history of S-X-[R_4]-[R_2] at second regions, where X is an attached molecule on the support surface. In the absence of a photoresist layer, the R_2 reagent may react differently in first and second regions depending on the product of the reactions initiated by R_1 and R_4. For example, suppose that R_4 removed a protective group from the only reactive group on X, and that X is inert to R_1. Suppose further that R_2 is capable of coupling to the reactive group. In this case, the R_2 reagent will selectively couple to X in the second regions, even in the absence of a patterned barrier layer. Conversely, previous reagents may make a particular region completely unreactive to additional reagents. For example, suppose R_1, added a protective group to a single reactive group on X, and R_4 added no such protective group. Again, application of R_2 will lead to selective coupling in second regions with or without a patterned barrier layer. These examples illustrate that identical sub-histories can lead to very different synthetic results.

d. Removal of Photoresist

The solvent profile of the photoinactive polymer allows suitable strippers to be readily identified by those of skill in the art. In the case of photoresists that proceed by a non-crosslinking mechanism, the final photoresist typically is stripped using solvents that solubilize the polymeric component. Such solvents are typically unreactive and cause no adverse changes in the organic molecules attached to the array surface. In preferred embodiments, a suitable stripping solution is selected from the group consisting of dimethylformamide (DMF), N-methylpyrrolidone (NMP), or dimethylacetamide (DMAC). Photoresists based on the following preferred polymers will typically be stripped by the indicated solvents:

polyethylene (low density)	halogenated hydrocarbons
polypropylene	chlorinated hydrocarbons
poly(di-n-butyl itaconate)	THF
polyacrylamide	morpholine, water
poly(vinyl alcohol)	water, DMF
poly(allyl alcohol)	methanol, THF
poly(chlorotrifluoroethylene)	CCl_4
poly(oxypropylidene)	DMF
poly(2,5-dimethoxy-1,4-phenyleneethylene)	bromoform
poly(oxy-1,4-phenyleneoxyisophthaloyl)	m-terphenyl
poly(1-butene-co-sulfur dioxide)	acetone
poly(imino(1-oxotrimethylene))	chloroacetic acid
poly(1,3,4-oxadiazoles)	DMSO
poly(dibenzoxazole)	m-cresol
poly(dithiazoles)	DMF
poly(pyromellitimides)	dimethylacetamide
poly(benzimidazoles)	DMSO
poly(dibenzimidazoles)	N-methylpyrrolidone
polyamic acids	N-methylpyrrolidone
polyimides	N-methylpyrrolidone

For photoresists based on photo-crosslinking, stripping solutions are required that cleave the crosslinked polymeric network, but do not adversely affect any organic molecules attached to the array surface. Such stripping solutions require agents which specifically cleave bonds in the polymeric network. For example, photoresists based on cross-linked polyvinyl alcohol may be selectively stripped using aqueous sodium periodate as long as the organic molecules attached to the array lack linkages comprising two or more —OH or ═O groups attached to adjacent carbon atoms. Other selectively cleavable linkages in the polymer will be readily apparent to those of skill in the art.

The stripping process should substantially remove the entire photoresist layer. In other words, as noted above, the photoresist should be sufficiently removed to permit a desired reaction between underlying molecules and a reagent. Such a reaction should proceed at a yield that is at least 50%, and more preferably at least 90% of the yield observed for similar molecules that have not previously been coated with photoresist. Reaction yields may be readily determined with and without photoresist using standard techniques appropriate for the reaction of interest (see Glossary phrase "substantial removal").

The above process (coating with photoresist, selective irradiation of photoresist, substantial removal of photoresist from irradiated regions, reaction of exposed molecules within irradiated regions and removal of the remaining photoresist) may be repeated as many times as desired to achieve synthesis of different molecules in discrete known regions. It will be apparent that, within each subsequent step, irradiation may be targeted to regions that are the same as in previous steps, to regions in separate locations, or to regions that overlap previous regions to varying degrees.

Solid phase synthesis (or other attachment methods) may be used to generate an arrangement of ligands (e.g., an array) on the porous coating in virtually any shape, as described above. In a preferred embodiment, the ligand groups are arranged as an array on the porous coating, with each group comprising substantially pure ligands with a known and unique chemical composition. In some embodiments, each group comprises substantially pure ligands with a known and unique reagent history (see "reagent history" in glossary). According to some embodiments, several ligands are intentionally provided within the same predefined volume so as to provide material for an initial screening for biological activity, after which the material within the predefined volume exhibiting significant binding is further evaluated.

Thus, the methods provided herein may be used to produce an array of nearly any desired organic compounds in discrete known regions. In preferred embodiments, an array comprises greater than 10, 100, 1,000, 10,000, 10^5 or 10^6 unique ligands attached to a surface in discrete known regions. Such an array may occupy a total area of less than

1 cm^2. Each region preferably occupies an area less than about 10^6 μm^2, more preferably, less than 10,000 μm^2 or 100 μm^2, and may, in some embodiments, encompass a single ligand molecule.

The methods described above illustrate the manual construction of representative articles. It will, of course, be appreciated that automated or semi-automated methods can be used. Articles comprising the porous coating bearing attached ligands can be created by the automated application of metal oxide dispersions, and the automated addition and removal of reagents by mounting the porous coating in an automated reactor system. Successive photoresist layers and masks can be applied manually or automatically.

Ligand Arrays

An important aspect of the invention as disclosed herein, is the discovery that it is possible to produce crack-free porous coatings at least 25 microns thick, which are continuous and substantially uniform in thickness. Such features are essential in ligand array applications, which require a large but uniform ligand density on a surface.

Arrays established on a porous coating as provided herein have particular advantages with regard to screening for ligand-receptor binding. For example, a porous array provides easily recognized landmarks to rapidly identify the location of ligands bound by a receptor. In some embodiments, porous array elements serve as landmarks for their own automated removal from the adhesive surface using, for example, robotics and machine vision. Removal allows ligands to be segregated into individual reaction vessels, detached from the porous support, and screened for ligand-receptor binding. In certain embodiments, the surface between porous elements provides a differential surface tension, such that an applied receptor segregates into individual nanodroplets. Each nanodroplet adheres to a separate porous element. The spatial segregation of nanodroplets prevents the mixing of ligands from other porous elements so that in situ ligand-receptor binding can be assayed for each individual ligand.

Other advantages of a patterned porous coatings include readily recognized landmarks to align successive masks during solid phase synthesis. Also, patterned porous coatings accommodate other microfabricated systems on the substrate surface, which in some embodiments may connect with the porous elements as part of, for example, a multifunctional biochip. Microfabricated systems which may connect with such porous coatings include, for example, amplification, separation, detection, reagent delivery and semiconductor systems. Preferably, such systems are relatively small, manufactured as described above using microfabrication methods. Other microfabricated systems that may be connected to a porous coating include electronic circuitry, capillary electrophoresis (see Woolley et al., *Proc. Natl. Acad. Sci. USA* 91:11348, 1994), PCR (see Wilding et al., *Clin. Chem.* 40:1815, 1994), signal detection (see Lamture et al., *Nucl. Acids Res.* 22:2121, 1994) and microfluidic manipulation (see Burns et al., *Proc. Natl. Acad. Sci. USA* 93:5556, 1996). In some embodiments, the high ligand surface density of porous arrays may provide sufficient material to function as reagents in arrays of enzymatic reactions, including arrays of amplification reactions such as, for example, polymerase chain reactions or PCR (see Mullis, U.S. Pat. No. 4,683,202 and Mullis et al., U.S. Pat. No. 4,683,195). Other microfabricated elements which may be connected to individual patterned porous coatings bearing attached ligands and other compounds will be apparent to those skilled in the art.

It will be apparent that the type of ligands that may be attached to a porous coating as provided herein is without restriction. In preferred embodiments, the ligands may include, for example, potential pharmacologic, pesticide, or herbicide candidates, drug analogues, or important biologic polymers including DNA, PNA, PENAM and other nucleobase polymers. It will be understood, however, that such polymer ligands represent only a subset of the ligands possible using the methods provided herein. The number of reagents in a reagent history may vary over a wide range, and preferably vary from 2 to 100.

Attached compounds may be of any size and may, for example, have molecular weights less than about 10^1 gram/mole, 10^2 gram/mole, 10^3 gram/mole, 10^4 gram/mole, 10^5 gram/mole, 10^6 gram/mole or 10^7 gram/mole. Each attached compound is preferably substantially pure and of known chemical composition or reagent history. Within certain embodiments, each discrete region contains a compound with a structure that is different from that of the compounds in every other discrete region. Within other embodiments, the same structure may appear in multiple discrete regions. For example, ligands may be present in two or more regions for purposes of redundancy. The percentage of compounds that share a structure may be very low, or may be greater than 10%, 50%, 70% or 90%.

The resulting arrangement of ligand groups, and the shape of the area occupied by each group can be essentially any size and any shape. For example, squares, ellipsoids, rectangles, triangles, circles, or portions thereof, along with irregular geometric shapes, may be utilized. Two-dimensional arrays are generally preferred.

Certain preferred ligands are nucleobase polymers. A nucleobase polymer is a polymer of nucleobases linked to a backbone. The backbone may be naturally occurring or non-naturally-occurring. Nucleobases linked to such a backbone may be naturally-occurring or non-naturally-occurring. Such nucleobase polymers may be capable of hybridizing specifically to particular nucleic acid sequences (e.g., antisense molecules). Besides resistance to degradative enzymes, some arrays of nucleobase polymers offer additional advantages. For example, PNA arrays provide for more rapid hybridization, greater specificity, more convenient hybridization conditions (i.e., hybridization of short probes at higher temperatures) and the ability to hybridize duplex DNA directly via DNA strand displacement and triplex formation.

A further advantage of many nucleobase polymers is the ability to penetrate the membranes of living cells. In embodiments employing nucleobase polymers capable of permeabilizing cell membranes, arrays as described herein can be used to modulate gene expression in an antisense manner. Within such embodiments, each nucleobase polymer of the array is detached from the substrate while in contact with one or more living cells, preferably using an enzyme-labile linker as described herein.

Nucleobases that may be incorporated into a nucleobase polymer include, for example, purine bases and pyrimidine bases, which may be naturally-occurring or analogs of naturally-occurring bases. A large variety of analogs have been described that exhibit properties that may be advantageous in particular array applications. For example, in some cases, it may be desirable to incorporate a nucleobase that binds non-specifically at a particular position. The nucleobase present in inosine is an example of such a non-specific analog. This can be used to incorporate degeneracy into nucleobase polymers at particular positions which might be particularly useful, for example, in targeting a closely related family of target nucleic acids that are homologous except for one or a few positions in their nucleobase

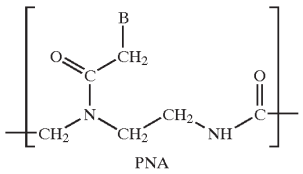
sequences. Inosine can pair with all four natural nucleobases, although the strength of binding varies: dC>dA>dG/T. Alternatively, the universal nucleobase 3-nitropyrrole-2' deoxynucleoside may be used to introduce degeneracy. In this strategy, the analog does not hybridize significantly to the other four natural nucleobases and makes up some of the duplex destabilization by acting as an intercalating agent.

Other types of modified nucleobases that may be of particular interest are those which enhance binding affinity. For example, diaminopurine can form three hydrogen bonds with thymine, whereas adenine and thymine form only two. Similarly, pyridopyrimidine nucleobases can be used in place of cytosine to provide stronger pairing with guanine.

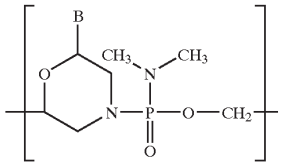
Nucleobases can also comprise any of a variety of "target receptor modifying groups". By way of illustration, nucleobases can function as cross-linking moieties. For example, 6-bromo-5,5-dimethoxyhexanohydrazide can be introduced into the C^4 position of cytidine to alkylate and thereby crosslink guanosine (see Summerton and Bartlett, *J. Mol. Biol.* 122:145, 1978). N^4, N^4-Ethano-5-methyl-cytosine can be used to similar effect (see Webb and Matteucci, *J. Am. Chem. Soc.* 108:2764, 1986 and Cowart et al., *Biochemistry* 28:1975, 1989).

A wide range of purine and pyrimidine analogs exhibiting various properties is known in the art (reviewed in Conholly, *Methods Enzymol.* 211:36, 1992; Lin and Brown, *Methods Mol. Biol.* 26:187, 1994 and Meyer, *Methods Mol. Biol.* 26:73, 1994). Such analogs include, for example, bromothymine, azaadenines and azaguanines. An exemplary but not exhaustive list of such analogs includes: 1-methyladenine, 1-methylguanine, 1-methylinosine, 1-methylpseudouracil, 2-methylthio-N-^{6}-isopentenyladenine, 2-thiocytosine, 2-methyladenine, 2-methylguanine, 2-thiouracil, 2,2-dimethylguanine, 2,6-diaminopurine-3-methylcytosine, 3-(3-amino-3-N-2-carboxypropyl)-uracil-4-acetylcytosine, 4-thiouracil, 5-fluorouracil, 5-iodouracil, 5-bromouracil, 5-methyluracil, 5-methyl-2-thiouracil, 5-methoxyaminomethyl-2-thiouracil, 5-chlorouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-methylaminomethyluracil, 5-carboxyhydroxylmethyluracil, 5-carboxymethylaminomethyluracil, 5-methoxyuracil, 5-methylcytosine, 7-methylguanine, 7-deazaguanine, 7-deazaadenine, β-D-mannoseylqueosine, β-D-galactosylqueosine, dihydrouracil, hypoxanthine, inosine, N-uracil-5-oxyacetic acid methylester, N^6-methyladenine, N^6-isopentenyladenine, pseudouracil, queosine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid and xanthine.

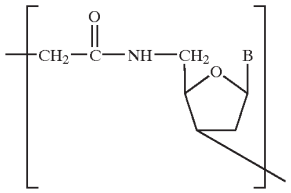
Representative examples of suitable nucleobase polymers include peptide nucleic acids (see Buchardt et al., PCT WO 92/20702 and Buchardt et al., U.S. Pat. No. 5,719,262), which offer a number of advantages over DNA including stronger binding independent of salt concentration (i.e., a higher T_m than a corresponding DNA probe), greater specificity of interaction, reduced hybridization times and resistance to environmental nucleases. Under low salt conditions, PNA binding is so energetically favorable that it binds duplex DNA directly by displacing one strand of the duplex. Other suitable nucleobase polymers include morpholino-based nucleobase polymers (see Summerton and Weller, U.S. Pat. No. 5,698,685; Summerton et al., U.S. Pat. No. 5,378,841 and Summerton and Weller, U.S. Pat. No. 5,185,444), peptide-based nucleic acid mimics or PENAMs (see Shah et al., U.S. Pat. No. 5,698,685), and polynucleosides with linkages comprising carbamate (see Stirchak and Summerton, *J. Org. Chem.* 52:4202, 1987), amide (see Lebreton et al., *Synlett. p.* 137, February), methylene (methylimino) (see Vasseur et al., *J. Am. Chem. Soc.* 114:4006, 1992), 3'-thioformacetal (see Jones et al., *J. Org. Chem.* 58:2983, 1993), sulfamate (see Huie and Trainor, U.S. Pat. No. 5,470,967) and others (see Swaminathan et al., U.S. Pat. No. 5,817,781 and Freier and Altmann, *Nucl. Acids Res.* 25:4429, 1997 and references cited therein). Particularly preferred nucleobase polymers contain repeating units as indicated below, where B is a naturally-occurring nucleobase or a non-naturally-occurring nucleobase:



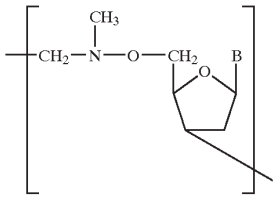
PNA



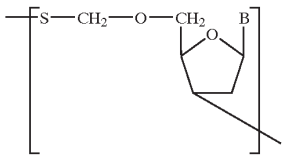
morpholino



amide-3



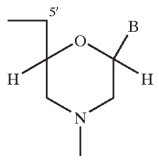
methylene(methylimino)



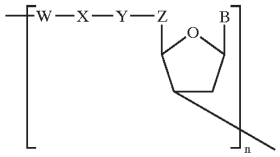
3'-thioformacetal

Other suitable nucleobase polymers will be readily apparent to those of skill in the art.

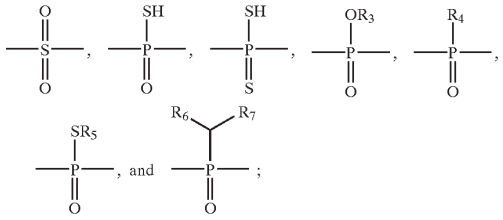
Additional representative nucleobase polymers include those comprising a morpholino subunit of the form:



wherein (i) the subunits are linked together by uncharged phosphorus-containing, chiral linkages, one to three atoms long, joining a morpholino nitrogen of one subunit to a 5', exocyclic carbon of an adjacent subunit, and (ii) B is a nucleobase. Other nucleobase polymers may comprise a repeating unit of the form:



wherein each W is independently selected from the group consisting of —CH_2—, —O—, —S—, —CH═, —CO— and —NR_1—, wherein R_1 is hydrogen or a spacer; each X is independently selected from the group consisting of —CH_2—, —O—, —S—, —CH═, ═CH—, ═N—, —CO—, —NR_2—,



(wherein R_2 is hydrogen or a spacer, R_3 is alkyl or a spacer, R_4 is alkyl, cyanoethyl or a spacer group, R_5 is hydrogen or a spacer, R_6 is hydrogen or a spacer group, and r_7 is hydrogen or a spacer); each Y is independently selected from the group consisting of —CH_2—, —O—, —S—, —CH≡, —CH═, ═CH—, ═N—, —CO— and —NR_8—, wherein R_8 is hydrogen or a spacer; each Z is independently selected from the group consisting of —CH_2—, —O—, —S—, ═CH—, —CO— and —NR_9—, wherein R_9 is hydrogen or a spacer; each B is independently selected from the group consisting of nucleobases; and each n is an independently selected integer ranging from 1 to 100. Other representative ligands include linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either α-, β-, or ω-amino acids, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polysiloxanes, polyimides and polyacetates.

Within preferred embodiments, an array comprises attached ligands that are resistant to degradative enzymes. In other words, at least 50% of the ligands should remain undegraded over a period of time sufficient to perform one or more useful assays in the presence of any degradative enzyme (i.e., nuclease or protease). Such arrays provide significant advantages, since they may be used in harsh environments, repetitively, with crude cell extracts or in any environment that might expose the ligand-array to the action of degradative enzymes. In applications that require the array be used to screen for the binding of nucleic acids, the resistant ligands are preferably nucleobase polymers with non-naturally occurring backbones.

Within one embodiment of a nucleobase polymer ligand-array, some of the nucleobase polymers may comprise at least one set of 2 to 10 different probes useful for interrogating the identity of a target nucleobase at a particular position in a reference sequence. One probe in a set is completely complementary to a 4 to 40 nucleotide portion that spans the reference sequence and the target nucleobase. The other probes are identical to the first probe, except that each comprises a different nucleobase substitution at the position of the target nucleobase (i.e., replacement of a particular nucleobase with a different nucleobase including nucleobase analogs, without altering the structure of the polymer backbone). Preferably, the nucleobase substitution will be centrally placed relative to the length of a probe, although this is not an absolute requirement. Contact of the array with the reference sequence will determine the identity of the target nucleobase by yielding the greatest amount of hybridization at the probe in a set which is completely complementary to the reference sequence, and lower amounts of hybridization at the probes in the set that are less than completely complementary. For example, if the reference sequence is labeled with a fluorescent label, then one may determine which probe in a set has the greatest amount of hybridization by determining which probe in the set has the strongest fluorescent signal. In particular, where non-set-containing arrays may have led to an ambiguous positive or negative signal for a particular target nucleobase detected in isolation, set-containing arrays facilitate correct recognition of the target nucleobase by providing side-by-side signal comparison for every possible target nucleobase. Preferred reference sequences for such arrays include, but are not limited to human immunodeficiency virus, human p53 gene, human CFTR gene, human factor V gene, human BRCA1 gene, human BRCA2 gene, a human leukocyte antigen and a human single nucleotide polymorphism.

The signal differential between correct and incorrect signals may be further increased through the use of a ligand-array comprising PNA nucleobase polymers. As noted above, PNA provides a greater specificity of interaction, with single nucleobase mismatches in PNA/DNA heteroduplexes being more destabilizing than the corresponding mismatches in DNA/DNA duplexes. For instance, a single mismatch in a PNA/DNA heteroduplex of length 15 lowers the T_m by an average of 15° C., whereas the T_m of the corresponding DNA/DNA duplex is lowered by an average of 11° C.

Although sufficient signal differentiation will usually be possible by employing sets comprising 4 probes, wherein each probe has either adenine (A), guanine (G), cytosine (C), or thymine (T) at the target position, in some embodiments it may be preferable to employ additional probes (i.e., up to a total of 10) comprising nucleobase analogues at the target position. Nucleobase analogues can be used that either stabilize or destabilize the hybridization of certain probes, and as a result, may clarify signals that would otherwise be ambiguous from probes containing only naturally occurring nucleobases. For example, suppose probes containing A and G at the target position gave about equal hybridization. Such a result would suggest two possibilities for the identity of the target nucleobase. The first possibility is that the target nucleobase is T, and the other hybridization signal represents

hybridization from a T/G mismatch. The second possibility is that the target nucleobase is C, and the other hybridization signal represents hybridization from a C/A mismatch. The correct possibility may be determined by including an additional probe in the set that contains the analog 2,6-diaminopurine at the target position. If the target nucleobase is T, the probe containing 2,6-diaminopurine will yield increased hybridization relative to the hybridization from probes containing A and G. Alternatively, if the target nucleobase is C, hybridization will be unchanged or decreased relative to the hybridization from probes containing A and G. Other nucleobase analogs for increasing the difference in binding energy between possible target nucleobases suitable for inclusion in a set will be apparent to those skilled in the art.

There is no restriction on the number of such sets that an array may comprise, except as dictated by the total number of probes on an array. Maximally, the total number of sets on an array will be one-half the number of probes, and is preferably less than 100,000 sets. Relative to the reference sequence, set probes may overlap one another by any number of nucleobases, or not at all. The number of target nucleobases that may be interrogated is also without particular restriction, being limited by the total number of target nucleobases in the reference sequence. Such set-containing arrays may be used, for example, to conveniently screen for single nucleotide polymorphisms (i.e., SNPs), variants of transplantation antigens (e.g., HLAs) and single nucleobase mutations such as occurs in genetic diseases (e.g., cystic fibrosis, factor V deficiency), drug resistant pathogens (e.g., HIV and bacteria), and neoplasia (e.g., p53 gene, BRCA1 gene, and BRCA2 genes).

According to a preferred embodiment, n sets of 2 to 4 probes, more preferably sets of 4 probes, each of length 1, will be used to interrogate n target nucleobases, where the reference sequence is n nucleobases in length. Thus, every nucleobase in a reference sequence may be interrogated with sets that collectively span the reference sequence. Set-containing arrays that interrogate the identity of every nucleobase in a sequence may be used, for example, to rapidly sequence a nucleic acid molecule. The nucleic acid molecule will comprise either a known reference sequence or a variant of a known reference sequence, wherein the variant contains one or more nucleotide substitutions at a frequency not greater than 2 per any (1+2) nucleotide stretch. For values of 1 ranging from 4 to 40, the variant will thus contain one or more substitutions at a frequency not greater than 2 per any 6 to 42 nucleotide stretch, respectively. At stretches where the frequency of substitution is greater than this limit, all probes will necessarily span more than one nucleotide substitution. This results in highly variable T_m values across different sets, leading to stringency conditions that are difficult to optimize.

Most preferably, set-containing arrays will contain sets comprised of nucleobase polymers that are resistant to degradative enzymes. Such articles have the significant advantage of being suitable for interrogating target nucleobases and sequencing nucleic acid molecules in a wide variety of harsh environments that contain degradative enzymes. Environments where it is desirable to perform such interrogation and sequencing, but where degradative enzymes are expected include the environments found in bodily samples such as blood, tissues, sputum, urine, and feces from both humans and animals. Other desirable harsh environments include food testing facilities, soil testing facilities, and waste water and sewage treatment plants. Other such desirable harsh environments will be readily apparent to those of skill in the art, as will the value and utility of such resistant articles in such environments.

Within other embodiments, an array may comprise ligands with attached target receptor modifying groups capable of affecting the interaction between the ligand and its target receptor, and/or affecting the target receptor itself. Examples of such modifying groups include labeling groups, intercalating groups, cleaving groups and other groups that reconform or bind to the receptor or modify the receptor. One type of modifying group that can be introduced into ligands is a nucleic acid intercalating group. A number of such intercalating groups are known in the art, many of which are acridine derivatives (see Helene and Thuong, *Genome* 31(1):413, 1989; Asseline and Thuong, *Nucleosides and Nucleotides* 10(1–3):359, 1991; Helene, *Anticancer Drug Des.* 6(6):569, 1991 and Wilson et al., *Biochemistry* 32(40):10614, 1993).

Another type of modifying group is a cross-linking group. Cross linking can be used to stabilize the interaction between a ligand and its target, which may be especially useful in achieving and stabilizing triple helix formation. Various approaches to the stabilization of triple helix formation include photochemical crosslinking (as described, for example, by Le Doan, *Nucleic Acids Res.* 15:7749, 1987 and Praseuth et al., *Proc. Natl. Acad. Sci. USA* 85:1349, 1988) and alkylation of the N7 of specific guanines in the target duplex (as described by Vlassov, *Gene* 72:313, 1988 and Fedorova et al. *FEBS Let.* 228:273, 1988).

Crosslinking can also be used to covalently link a new molecular structure, attached to a ligand, to a particular location within a target receptor. Thus, for example, a label attached to a ligand could be linked to a particular location within a receptor targeted by the ligand. Such labels could be photo-induced cross-linking agents, such as psoralen, coumarin, ellipticine and their derivatives (see Perrouault et al., *Nature* 344:358, 1990; Le Doan et al., *Antisense Res. Dev.* 1(1):43, 1991; Miller, *Methods Enzymol.* 211:54, 1992; Havre et al., *Proc. Natl. Acad. Sci. USA* 90(16):7879, 1993; and Rajagopalan et al., *J. Biol. Chem.* 268(19):14230, 1993).

Other labels that do not involve a cross-linking group may be used. A number of such labeling groups are known in the art (see Haralambidis et al., *Nucleic Acids Res.* 18(3):501, 1990; Strobel et al., *Bioconjug Chem.* 2(2):89, 1991; and Durrant and Chadwick, *Methods Mol. Biol.* 28(141):141, 1994). Such groups may be used, for example, to label particular sequences in a nucleic acid, which is useful in efforts to map and sequence various genomes.

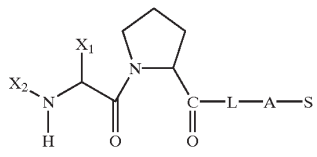
Other modifying groups that can be introduced into a ligand array are nucleic acid alkylating agents. A number of such alkylating groups are known in the art. For example, such groups include N-mustards as reactive alkylating compounds (see Lee et al., *J. Med. Chem.* 37(8):1208, 1994), porphyrins (see Boutorine et al., *Bioconjug. Chem.* 1(5):350, 1990 and Brossalina et al., *Antisense Res. Dev.* 1(3):229, 1991), psoralens as photochemical activatable agents (see Bhan and Miller, *Bioconjug Chem.* 1(1):82, 1990 and Miller, *Methods Enzymol.* 211:54, 1992) and quinones as inducible alkylating agents (see Chatterjee and Rokita, *J. Am. Chem. Soc.* 112:9387, 1990).

Still further modifying groups that can be introduced into a ligand are nucleic acid cleaving groups. There are a number of cleaving groups that can be used to allow a ligand in an array to act as an artificial sequence-specific nuclease, which have been described in the art (see Strobel and Dervan, *Methods Enzymol.* 21:309, 1992; Sigman and Chen, *Annu. Rev. Biochem.* 59:207, 1990; Jayasena and Johnston, *Proc. Natl. Acad. Sci. USA* 89:3526, 1992; Podhajska et al.,

Methods Enzymol., 216:303, 1992; Huber, *Faseb J.* 7(14) :1367, 1993; Kappen and Goldberg, *Science* 261:1319, 1993; Sigman et al., *Nature* 363:474, 1993; and Shimzu et al., *Biochemistry* 33(2):606, 1994). The following representative approaches are intended as an illustrative, not an exhaustive, list of cleaving groups. In one approach, iron (III) EDTA is used as a cleaving group which generates free radicals under appropriate redox conditions as described by Moser and Dervan, *Science* 238:645, 1987. Other redox-activated transition metal cleaving groups include complexes of o-phenanthroline-Cu(I) (introduced by Francois et al., *Proc. Natl. Acad. Sci. USA* 86:9702, 1989) and porphyrins-Fe(II) (see Le Doan, *Nucleic Acids Res.* 15:8643, 1987). These systems may be more useful in vitro, where redox activation is more readily controlled. Another alternative is photochemical cleavage as described by Perrouault et al., *Nature* 344:358, 1990. Still another approach is to incorporate as a cleaving group a relatively non-specific nuclease such as DNasel or staphylococcal nuclease and effectively convert it into a specific endonuclease by conjugation to ligands in the array (see Corey and Schultz, *Science* 238:1401, 1987 and Pei et al., *Proc. Natl. Acad. Sci. USA* 87(24):9858, 1990). In this embodiment, the nuclease-resistance of the nucleobase polymers in the present invention is a major advantage. Yet another possible approach to cleaving target nucleic acids is to incorporate a ribozyme into the ligand array (see Haseloff and Gerlach, *Nature* 334:585, 1988; and Van and Hecht, *Adv. Inorg. Biochem* 9:1, 1994).

A modifying group can be incorporated anywhere within a ligand. However, there are a number of general considerations that should guide selection of a particular group and location. The most significant consideration is that the group should not be introduced into a position that is likely to prevent sufficient hybridization between the ligands and the target receptor. Thus, while small modifying groups can be accommodated within the region of hybridization, larger groups may be better accommodated outside of the region of hybridization. Even large modifying groups such as nuclease enzymes can be attached to terminal regions of nucleobase polymers. In some cases, the nature of the interaction between the modifying group will dictate favorable positions within the ligand. Moreover, molecular modeling can be used to anticipate favorable positions for the incorporation of such groups.

In certain embodiments, an array may comprise ligands that are drug candidates, preferably greater than 500 different drug candidates. Each drug candidate is preferably attached to the surface in quantities sufficient for screening using functional assays. Certain such reagents give rise to arrays of enaprilat analogues having the formula:



wherein S is the surface, A is aminopropyltriethoxysilane, L is a divalent linker molecule, X_1 is a monovalent organic group or hydrogen, and X_2 is a monovalent organic group or hydrogen. X_1 and X_2 may, within certain embodiments, further comprise acid labile protecting groups (i.e., removed by an acid, usually TFA or trifluoroacetic acid), such as tert-butoxycarbonyl (t-Boc), benzhydryloxycarbonyl (Bhoc), trimethylsilyl, t-butyl, phenoxyethyl or tetrahydropyranyl groups.

According to some embodiments, multiple ligands are intentionally provided within the same known discrete region so as to provide material for an initial receptor binding screen, after which the material within the predefined region exhibiting significant binding is further evaluated. In alternative embodiments, each known discrete region is recessed beneath the surface in, for example, a well. Each well is preferably of substantially similar dimensions to the region within it. In other embodiments, the surface between array elements provides a differential surface tension, such that an applied liquid segregates into individual droplets over each known discrete region. In some embodiments, the liquid contains an assay mixture capable of detecting binding of receptor in situ. The spatial segregation of droplets prevents the mixing of detached ligands from individual array elements. The differential surface tension may be provided by one or more organosilanes attached in a specific pattern to the surface.

Using patterned photoresist layers to make regions more or less reactive to subsequently added reagents can be used advantageously as a method for adding regional selectivity to reagents that are ordinarily incompatible with the patterned photoresist layer. For example, the synthesis of PNA uses the preferred solvents 1-methyl-2-pyrrolidinone and dimethylformamide in monomer coupling reactions. Although the polyamide photoresist is resistant to numerous solvents, it is particularly sensitive to degradation by amide solvents. This limitation is overcome by removing protective groups from the elongating end of attached PNA molecules in selected regions using a patterned photoresist layer and a compatible deprotection reagent. PNA molecules in those regions then become reactive to monomer coupling, while the remaining protected PNA molecules are unreactive. The photoresist is then stripped, and the coupling solution applied to the porous surface. Monomer couples selectively to those regions where protective groups were removed, even in the absence of a patterned barrier layer.

In some embodiments, array elements may connect with other microfabricated systems on the substrate surface as part of, for example, a multi-functional biochip. Microfabricated systems which may connect with array elements include, for example, amplification, separation, detection, reagent delivery or semiconductor systems. Preferably, such systems will be relatively small, manufactured using microfabrication methods. For example, microfabricated systems which might be connected to individual array elements include electronic circuitry, capillary electrophoresis (see Woolley et al., *Proc. Natl. Acad. Sci. USA* 91:11348, 1994), PCR (see Wilding et al., *Clin. Chem.* 40:1815, 1994), signal detection (see Lamture et al., *Nucl. Acids Res.* 22:2121, 1994), and microfluidic manipulation (see Burns et al., *Proc. Natl. Acad. Sci. USA* 93:5556, 1996). Such systems may operate in direct connection with an array element bearing certain ligands.

Arrays of Nucleobase Polymers Resistant to Degradative Enzymes

As noted above, certain preferred arrays comprise nucleobase polymer ligands that are resistant to degradation by nucleases and proteases. Such arrays may be prepared using the methods provided above; the following illustrations are provided for exemplary purposes only. It will be apparent to those of skill in the art that articles comprising a support bearing arrays of other nucleobase polymers may be readily made using essentially identical chemistry as for the nucleobase polymers described in detail. It will also be apparent that, although the following illustrations describe manual array construction, automated or semi-automated methods

could be used. In particular, the application of photoresist a patterned irradiation, and addition and removal of reagents may be readily automated by those of ordinary skill in the art.

Representative PNA Array

A peptide nucleic acid (PNA) array contains ligands that comprise a backbone of repeating units of N-(2-aminoethyl)-glycine linked by amide bonds, with the bases attached to the backbone by methylene carbonyl linkages. If it is desired to synthesize all 16 possible reagent histories for a PNA dimer N_1N_2 using four monomers (denoted A, C, G and T) for N_1 and N_2, a square region of the support surface can be divided conceptually into a 4×4 array of 16 boxes. For illustrative purposes, it is assumed that the monomer units are the only reagents needed to form the desired PNA molecules, although it will be understood that PNA synthesis requires other reagents such as activation, washing, capping, and deblock reagents as provided in the teachings of the prior art (see Egholm et al., *J. Am. Chem. Soc.* 114:1895, 1992; Coull et al. PCT WO 96/40685; Buchardt et al., PCT WO 92/20702 and Buchardt et al., U.S. Pat. No. 5,719,262). The N_1 reagents are applied to the four vertical columns of the conceptual array using a positive photoresist. The first photoresist barrier exposes the left-most column of boxes, where A is applied. The second photoresist barrier exposes the next column, where G is applied; followed by a third photoresist barrier, for the C column; and a final photoresist barrier that exposes the right-most column, for T. The first, second, third, and fourth photoresist barriers may be irradiated with a single mask translated to different column locations, or four individual masks represented by the following patterns:

$$m_1 = \begin{matrix} 1&0&0&0\\ 1&0&0&0\\ 1&0&0&0\\ 1&0&0&0 \end{matrix} \quad m_2 = \begin{matrix} 0&1&0&0\\ 0&1&0&0\\ 0&1&0&0\\ 0&1&0&0 \end{matrix} \quad m_3 = \begin{matrix} 0&0&1&0\\ 0&0&1&0\\ 0&0&1&0\\ 0&0&1&0 \end{matrix}$$

$$m_4 = \begin{matrix} 0&0&0&1\\ 0&0&0&1\\ 0&0&0&1\\ 0&0&0&1 \end{matrix}$$

where digits correspond to array elements, and a "1" represents a transparent mask region and a "0" represents an opaque mask region. The process is repeated in the horizontal direction for the N_2 reagents. This time, the A, G, C, and T monomers are sequentially applied using photoresist barriers that expose the four horizontal rows of the conceptual array. The fifth, sixth, seventh, and eighth photoresist barriers may be irradiated with a single mask translated to different row locations, or four individual masks represented by the following patterns:

$$m_5 = \begin{matrix} 1&1&1&1\\ 0&0&0&0\\ 0&0&0&0\\ 0&0&0&0 \end{matrix} \quad m_6 = \begin{matrix} 0&0&0&0\\ 1&1&1&1\\ 0&0&0&0\\ 0&0&0&0 \end{matrix} \quad m_7 = \begin{matrix} 0&0&0&0\\ 0&0&0&0\\ 1&1&1&1\\ 0&0&0&0 \end{matrix}$$

$$m_8 = \begin{matrix} 0&0&0&0\\ 0&0&0&0\\ 0&0&0&0\\ 1&1&1&1 \end{matrix}$$

The resulting substrate contains all 16 possible reagent histories placed as represented in Table III:

TABLE III

	columns			
rows	S-[A]-[A]	S-[G]-[A]	S-[C]-[A]	S-[T]-[A]
	S-[A]-[G]	S-[G]-[G]	S-[C]-[G]	S-[T]-[G]
	S-[A]-[C]	S-[G]-[C]	S-[C]-[C]	S-[T]-[C]
	S-[A]-[T]	S-[G]-[T]	S-[C]-[T]	S-[T]-[T]

In this illustration, the N_1 reagents couple to the support "S", and the N_2 reagents couple to the already attached N_1 reagents. As such, the reagent histories predict polymer formation at each array element, and the sequence composition of each polymer.

The preparation of PNA arrays further provides an example of the use of patterned photoresists for solid phase synthesis reactions that employ reagents which degrade the photoresist material. Although the polyamide photoresists described herein are resistant to numerous solvents, such photoresists can be sensitive to degradation by N-alkyl amide solvents, such as 1-methyl-2-pyrrolidinone and dimethylformamide, which are commonly used in PNA synthesis. This limitation can be overcome through the use of protective groups on the elongating end of the attached PNA molecules. Such protective groups can be removed in selected regions using a patterned photoresist layer and a compatible deprotection reagent. PNA molecules in those regions then become reactive to monomer coupling, while the remaining protected PNA molecules are unreactive. The photoresist is stripped after removal of protective groups, and the coupling solution is applied to the surface. Monomer couples selectively to those regions where protective groups were removed, even in the absence of a patterned barrier layer.

As a further illustration, suppose it is desired to synthesize all 16 possible PNA dimers using Fmoc protective groups (Fmoc: fluorenylmethyloxycarbonyl, a base-labile amino-protecting group removed under nonhydrolytic conditions). As before, the support surface is divided conceptually into a 4×4 array, and the four monomer units are denoted by Fmoc-A-OH, Fmoc-G-OH, Fmoc-C—OH, and Fmoc-T-OH. Again, it is assumed for illustrative purposes that the desired PNA may be formed using only monomer units and a deprotectant as reagents. A representative deprotection reagent compatible with the polyamide photoresist comprises, for example, 20% piperidine in toluene. The support surface bears Fmoc protected linker molecules designated as P-L-Fmoc. The Fmoc groups are selectively removed from the four vertical columns of the conceptual array. The first photoresist barrier exposes the left-most column Contact with deprotection reagent removes Fmoc from the left-most column of linker molecules. The photoresist is stripped, and the A monomer is applied to the entire array surface for 30 to 40 minutes using the representative coupling solution shown in Table IV.

TABLE IV

Representative Coupling Solution	
80 μl	215 mM Fmoc-monomer-OH in NMP
80 μl	181 mM HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate)
80 μl	300 mM 2,6-lutidine and 200 mM DIPEA (N,N-diisopropylethylamine) in DMF

The second photoresist barrier exposes the next column, where Fmoc is removed. The photoresist is stripped, and the G monomer applied. This cycle is repeated for the third

photoresist barrier resulting in Fmoc removal and coupling of C to the third column. A final photoresist barrier exposes the right-most column, and Fmoc is removed followed by stripping and T coupling. The process is repeated in the horizontal direction with the photoresist barriers allowing exposure of horizontal rows, and coupling of monomers to already attached monomers. The photoresist barriers are irradiated with 8 individual masks as described above by the patterns m_1 through m_8.

Although this method produces the same ligands as shown in Table III, the reagent histories are very different, as shown in Table V. In contrast to Table III, each reagent history in Table V contains every added monomer reagent, since every surface element was contacted by every monomer reagent. Specific coupling to an element occurred when deprotection preceded the addition of monomer. Accordingly, the monomer that follows the deprotection reagent in the reagent history couples to the attached linker if it is from the first set of 4 monomers, and couples to attached monomer if it is from the second set of 4 monomers.

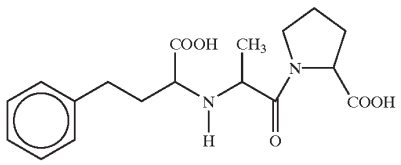
TABLE V

columns			
P-L-pip-[A]-[G]-[C]-[T]-pip-[A]-[G]-[C]-[T]	P-L-[A]-pip-[G]-[C]-[T]-pip-[A]-[G]-[C]-[T]	P-L-[A]-[G]-pip-[C]-[T]-pip-[A]-[G]-[C]-[T]	P-L-[A]-[G]-[C]-pip-[T]-pip-[A]-[G]-[C]-[T]
P-L-pip-[A]-[G]-[C]-[T]-[A]-pip-[G]-[C]-[T]	P-L-[A]-pip-[G]-[C]-[T]-[A]-pip-[G]-[C]-[T]	P-L-[A]-[G]-pip-[C]-[T]-[A]-pip-[G]-[C]-[T]	P-L-[A]-[G]-[C]-pip-[T]-[A]-pip-[G]-[C]-[T]
P-L-pip-[A]-[G]-[C]-[T]-[A]-[G]-pip-[C]-[T]	P-L-[A]-pip-[G]-[C]-[T]-[A]-[G]-pip-[C]-[T]	P-L-[A]-[G]-pip-[C]-[T]-[A]-[G]-pip-[C]-[T]	P-L-[A]-[G]-[C]-pip-[T]-[A]-[G]-pip-[C]-[T]
P-L-pip-[A]-[G]-[C]-[T]-[A]-[G]-[C]-pip-[T]	P-L-[A]-pip-[G]-[C]-[T]-[A]-[G]-[C]-pip-[T]	P-L-[A]-[G]-pip-[C]-[T]-[A]-[G]-[C]-pip-[T]	P-L-[A]-[G]-[C]-pip-[T]-[A]-[G]-[C]-pip-[T]

"pip" indicates piperidine in toluene, use "deprotection reagent".

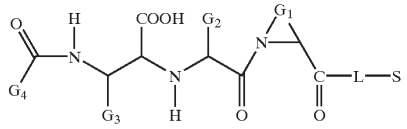
Representative Array of Enaprilat Analogues

As an illustration of a method for producing a support bearing a drug candidate array, 625 enalaprilat analogues may be synthesized in an array. Enalaprilat is one of a class of antihypertensive drugs that bind angiotensin-converting enzyme (ACE) and inhibit its dipeptidase activity. ACE generates the powerful vasoconstrictor substance angiotensin II by removing the C-terminal dipeptide from the precursor decapeptide angiotensin I. Enalaprilat is a dipeptide analogue with the following formula:



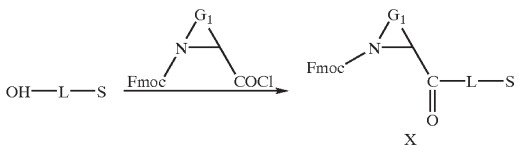
Enalaprilat is a carboxyalkyldipeptide transition-state inhibitor with the $CHCO_2H$ and NH groups mimicking the transition state-like geometry attained at the scissile peptide bond of angiotensin I (see Patchett et al., *Science* 288:280, 1980). Screening of enalaprilat analogues may be used to identify ACE inhibitors with improved potency, bioavailability, half-life, or side-effect profile.

The 625 analogues of enalaprilat have the following general formula:



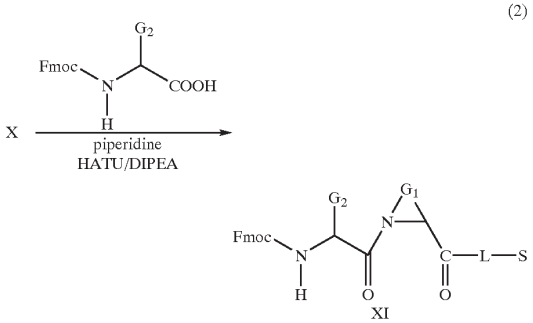
where S is the substrate, L is a linker, and G_1, G_2, G_3, and G_4 are monovalent organic groups or hydrogen. The G_1 group is in some embodiments attached to either nitrogen or carbon but not to both. Each analogue is synthesized directly on the support by 4 sequential couplings of reagents, with each successive coupling adding G_1, G_2, G_3, and G_4. In this example, each G_n group can assume 1 of 5 different compositions designated G_{na}, G_{nb}, G_{nc}, G_{nd}, and G_{ne}, where n=1 to 5. By using a series of patterned photoresist layers to combinatorially direct each reagent composition to predefined regions of the support, a total of 5^4 or 625 different analogues are possible using a total of 20 couplings. Each of the four coupling reactions used to synthesize a given analogue XIII, are shown below:

(1)



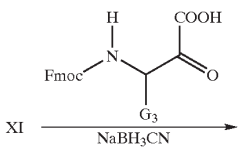
X

(2)

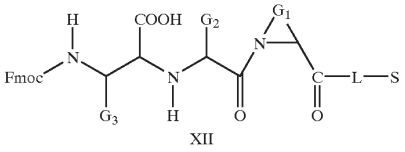


XI

(3)

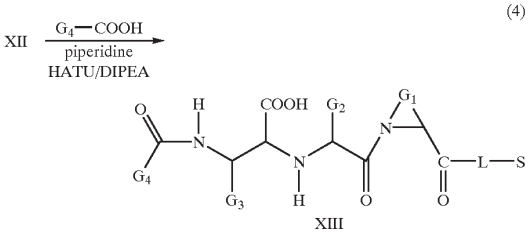


-continued



XII

(4)



XIII

To place these 625 analogues on the support using solid-phase synthesis and a polyamide photoresist, it is convenient to divide the surface into a 25×25 array of 625 boxes. As shown in Table VI, the array synthesis is completed using 20 reaction cycles comprising 20 patterned photoresist layers and 5 applications of each of the above coupling reactions. For each G_n in the array, five patterned photoresist layers direct the application of compositions designated G_{na}, G_{nb}, G_{nc}, G_{nd}, and G_{ne} by either blocking reagents directly, or making regions more reactive to reagents. Each photoresist layer is patterned with an individual mask comprising a pattern of transparent rows or columns corresponding to the rows or columns in the 25×25 array. Each pattern is indicated by a condensed notation in Table VI. This notation represents every mask pattern by a column or row cross-section. For example, the pattern notation for cycle indicates a mask with transparent columns corresponding to every fifth column in the 25×25 array. In cycle 20, the pattern notation is the same, but the mask type indicates that the pattern notation refers to a mask where every fifth row is transparent. In most embodiments, synthesis of the analogue array is followed by the removal of protecting groups from one or more G_n groups.

TABLE VI

Cycle	Reaction	Composition	Mask Type	Mask Pattern
1	1	G_{1a}	column	11111000000000000000000000
2	1	G_{1b}	column	00000111110000000000000000
3	1	G_{1c}	column	00000000001111100000000000
4	1	G_{1d}	column	00000000000000001111100000
5	1	G_{1e}	column	00000000000000000000011111
6	2	G_{2a}	row	11111000000000000000000000
7	2	G_{2b}	row	00000111110000000000000000
8	2	G_{2c}	TOW	00000000001111100000000000
9	2	G_{2d}	row	00000000000000001111100000
10	2	G_{2e}	row	00000000000000000000011111
11	3	G_{3a}	column	10000100001000010000100000
12	3	G_{3b}	column	01000010000100001000010000
13	3	G_{3c}	column	00100001000010000100001000
14	3	G_{3d}	column	00010000100001000010000100
15	3	G_{3e}	column	00001000010000100001000010
16	4	G_{4a}	row	10000100001000010000100000
17	4	G_{4b}	row	01000010000100001000010000
18	4	G_{4c}	row	00010000100001000010000100
19	4	G_{4d}	row	00010000100001000010000100
20	4	G_{4e}	row	00001000010000100001000010

In some embodiments, each G_n group is selected from 1 of 10 different compositions. By forming every G_n combination, 10^4 or 10,000 analogues are synthesized in a total of 40 cycles. Other agents with known or potential pharmacologic activity available by combinatorial solid-phase synthesis include, for example, analogues of benzodiazepine, sulfonamide, hydantoin, miconazole, dihydropyridone, pyrazolone, pyrimidine, quinazoline, quinazolinone, oligocarbamates, peptoids, and peptidyl phosphonates. Accordingly, it will be appreciated by those of skill in the art that the above method can be used for the parallel production of supports bearing thousands or millions of drug candidates and other compounds using barrier layers and the photolithographic techniques disclosed herein.

Use of Arrays for Ligand-Receptor Binding Assays

Articles comprising one or more porous coatings as described herein may be used to screen for ligand-receptor binding. For example, such arrays can be used to determine peptide and nucleobase sequences that bind to proteins or nucleic acids, identify epitopes recognized by antibodies, evaluate a variety of drugs and metabolites for clinical and diagnostic applications, and screen small-molecule libraries for novel drugs, pesticides, or herbicides, as well as combinations of the above. In some embodiments where the ligand and receptor are both polymers, the sequence of the polymer at the locations where the receptor binding is detected may be used to determine all or part of a sequence which is complementary to the receptor. Of course, it is also possible to screen for ligand-receptor binding using receptor arrays, rather than ligand-arrays, using the methods provided herein.

To use a ligand-array to identify ligands that bind a specific receptor, the array is first contacted with a receptor of interest under conditions and for a time sufficient to permit receptor-ligand interaction. Following such contact, any of a variety of methods may be used to determine whether any ligands attached to the array specifically bind the receptor.

As noted above, there are a variety of molecules that may be used as receptors within such assays, including nucleic acid molecules, polypeptides, peptides, PNA, enzymes, enzyme cofactors, lectins, sugars, polysaccharides, antibodies, cell receptors, phospholipid vesicles, or any one of a variety of other receptors. Alternatively, a receptor may be a biological structure such as a cell, cellular membrane or organelle. A receptor may bind with zero, one or more ligands on the array. In some embodiments, a receptor may be from blood obtained from either healthy or diseased subjects, and screening an array for binding by the receptor may have diagnostic applications.

A receptor may be contacted with an array by placing an aliquot of a receptor solution directly on the array. Optionally, a microscope cover-slip is then placed on the receptor solution. In other embodiments, a receptor solution may be applied while the array is mounted to a reactor system as shown in FIG. **3** by circulating the receptor solution through inlet and outlet ports. Alternatively, an entire array may be immersed in a receptor solution. In addition to receptor, receptor solutions may contain one or more buffers, salts, protein, nucleic acid, detergents, cofactors, polyelectrolytes and/or other such materials necessary for a particular receptor to bind ligand. Such binding adjuvants are well known in the art. Representative DNA receptor and antibody receptor solutions which may be

utilized to screen the support for ligand-receptor binding are shown in Table VII.

TABLE VII

DNA Receptor Solution	Antibody Receptor Solution
10 nM to 10 μM labeled DNA	0.1 to 4.0 μg/ml antibody
66 mM NaH_2PO_4/Na_2HPO_4	0.2M NaH_2PO_4/Na_2HPO_4
1M NaCl	150 mM NaCl
6 mM EDTA	1% bovine serum albumin
0.05% Triton X-100 ™	0.5% Tween-20 ™

During contact, it may be important to maintain a specific temperature of the array. For example, temperature can influence the stringency of DNA, PNA, and other nucleobase polymer interactions such that specific binding to particular array elements will only be observed in a narrow temperature range. In other cases, a particular temperature may be required for a receptor to either adopt a needed conformation, or avoid thermal denaturation. An optimal temperature for performing an assay may be readily determined by those of ordinary skill in the art.

If the temperature range for specific binding overlaps for all array elements, then a discrete temperature may be identified that is suitable to simultaneously screen for ligand-receptor binding at all array elements. Alternatively, if the temperature range for specific binding does not overlap for some array elements, then screening for ligand-receptor binding may have to be performed at multiple discrete temperatures. In some embodiments, ligand-receptor binding may have to be performed over a temperature gradient that samples all temperatures between two discrete temperatures. Screening for ligand-receptor binding at a plurality of temperatures within a temperature gradient is particularly useful for arrays whose elements vary widely with respect to T_m and stringency.

Methods for maintaining the ligand-derivatized support at a particular temperature include, for example, placing the support in contact with a heating block, thermo-electric (Peltier) device, heated water bath, convection oven, refrigerator, freezer, or temperature controlled reactor system. In some embodiments, the substrate is mounted on a microscope stage that contains an aqueous gel within its interior chilled to a specific temperature. Other methods for controlling the temperature of the ligand-derivatized support during contact with a receptor will be apparent to those skilled in the art.

Methods for detecting binding include the detection of a marker that permits determination of the location of bound receptor on the array. Suitable markers are well known in the art, and include radionuclides and fluorescent molecules. Markers may indicate the presence of ligand-receptor pairs by producing, for example, a differential color, absorption of electromagnetic radiation, optical interference, electric conduction, radioactive decay, fluorescence, chemiluminescence, phosphorescence, or a molecular shape detectable by scanning tunneling microscopy (STM) or atomic force microscopy (AFM), either by themselves or via other covalently and non-covalently linked molecules, labels, nuclear isotopes, antibodies, or enzymes. In some embodiments the ligand-receptor pair may produce a phenotypic change including, for example, cessation of cell growth, initiation of cell growth, apoptosis or cellular differentiation. Other methods of locating and visualizing ligand-receptor pairs will be apparent to those skilled in the art.

A ligand-array may be exposed only to a labeled receptor. Alternatively, an array may be exposed to a first, unlabeled receptor of interest and, thereafter, exposed to a labeled receptor-specific recognition element, which is, for example, an antibody. Such a process provides for additional amplification of signal during detection. In yet another embodiment, a multi-labeling scheme may be employed whereby the ligand-derivatized support is exposed to several different receptors, each coupled to a different label or combination of labels. A set of images, each representing the surface density of a particular label can be generated using spectral deconvolution methods well known in the art. Such multi-labeling strategies have a variety of uses. For example, the microenvironment of the sample may be examined using special labels whose spectral properties are sensitive to some physical property of interest. In this manner, pH, dielectric constant, physical orientation, and translational and/or rotational mobility may be determined.

In a preferred embodiment using a porous array, the location of bound receptor on the array is determined by detecting fluorescence with a conventional charge-coupled device, a conventional film-based camera, or by visual inspection using fluorescence microscopy. One advantage of a porous support is an increased ligand surface density, such that imaging of bound receptors is both rapid and economical using standard equipment.

In other embodiments, an indicator compound is added that indirectly detects ligand-receptor binding. An indicator compound refers to a compound that has a detectable property in the presence of a receptor that is different when the receptor is bound by a ligand. Such detectable properties include color, light absorbance, light transmission, fluorescence, fluorescence resonance energy transfer, fluorescence polarization, phosphorescence, catalytic activity, molecular weight, charge, density, melting point, chromatographic mobility, turbidity, electrophoretic mobility, mass spectrum, ultraviolet spectrum, infrared spectrum, nuclear magnetic resonance spectrum, elemental composition and X-ray diffraction. In one embodiment, the indicator compound furylacryloylphenyalanylglycylglycine (FAPGG) is used to detect binding of angiotensin converting enzyme (ACE) by an array of enalaprilat analogues. Hydrolysis of FAPGG by ACE results in a decrease in absorbance at 328 nm. The decrease in absorbance is attenuated if ACE is bound by an enalaprilat analogue. Other indicator compounds will be readily apparent to those skilled in the art.

The signal-to-noise ratio of the assays provided herein is sufficiently high that the relative binding affinity of receptors to a variety of support-bound ligands can be determined. A receptor may bind to several ligands in an array, but may bind much more strongly to some ligands than others. Strong binding affinity will be evidenced herein by a strong fluorescent signal since many receptor molecules will bind in a region of a strongly bound ligand. Conversely, a weak binding affinity will be evidenced by a weak fluorescent signal due to the relatively small number of receptor molecules which bind in a particular region of the support having a ligand with a weak binding affinity for the receptor. Consequently, it is possible to determine relative binding avidity of a ligand herein by way of the intensity of a fluorescent signal in a region containing that ligand. In preferred embodiments, using a porous support as described above, this can be performed economically and with standard equipment. Semiquantitative data on affinities may be obtained by the inclusion of one or more ligands with known binding constants.

Depending on the application, ligand-receptor binding assays may be performed on attached or detached ligands. In preferred embodiments where ligands are biologic polymers

such as DNA or PNA, ligands are screened for receptor binding while attached to the substrate surface. In preferred embodiments where ligands with potential pharmacologic activity are being screened, ligands are screened after they are detached from the substrate surface. Such assays permit the detection of ligand-receptor binding that may be sterically restricted by attachment of the ligand to the support. In such screens, it is preferred that the detached ligands have a local concentration of at least 10 μM, thereby allowing identification of ligands with low to moderate binding affinities. This may be accomplished, for example, using a porous coating as described herein, in which the concentration of ligand in the porous coating typically exceeds 10 μM, and in some embodiments is greater than 2 mM, 10 mM, 50 mM, or 200 mM. In preferred embodiments, ligands are detached without losing their positional information, since array position determines the reagent history and preferably the composition of each detached ligand. Without positional information, screening is less straightforward requiring deconvolution of pooled ligands via iterative syntheses, or analysis of orthogonally synthesized encoded-tags (reviewed by Gordon et al., *J. Med. Chem.* 37:1385, 1994). All or a portion of the ligands may be detached from the surface simultaneously. Preferably, at least 50,% of the ligands are detached in a binding assay performed using detached ligands.

One method for maintaining positional information for ligands bound to a porous array involves separating known porous layers from the surface, and segregating each of them to a known reaction vessel. With each porous layer appropriately segregated, the ligands are detached from each porous layer and screened for ligand-receptor binding individually. Preferably, the separating and segregating processes are performed automatically using, for example, robotics and machine vision. In some embodiments, the separation of the porous layer from the adhesive surface may be in response to the local and selective application of, for example, light, heat, ultrasonic radiation, solvent, magnetism, vacuum, abrasion, adhesion, scraping, high-pressure liquid streams, laser radiation, or cutting. In other embodiments, the separating process may involve a release layer sandwiched between each porous layer and the adhesive surface. The release layer may affect separation of the porous layer from the adhesive surface in response to the local and selective application of any of the above conditions. In one embodiment, porous layers are sliced off the surface after the application of a polymeric binder to prevent fragmentation of the layers.

Another method for maintaining positional information involves connecting ligands to the solid-support via photocleavable linkers (e.g., linkers that are cleaved upon exposure to a particular ultraviolet, visible or infrared wavelength), base-labile linkers, acid-labile linkers or linkers that comprise a recognition sequence that is cleaved by an enzyme. Exposing such cleavable linkers to acid or base in the vapor-phase (e.g., trifluoroacetic acid or ammonia vapor), to light, or to cells having a cell surface enzyme that cleaves a linker allows separated ligands to remain co-localized with their site of attachment and/or synthesis on the array (see Quillan et al., *Proc. Natl. Acad. Sci. USA* 92:2894, 1995; and Bray et al., *Tetrahedron Lett.* 32:6163, 1991). Screening for ligand-receptor binding may then be performed by either removing individual detached ligand groups from the support (e.g., manually or by robotics), or more preferably, by performing an in situ assay for ligand-receptor binding (see You et al., *Chemistry & Biology* 4:969, 1997; and Schullek et al., *Analytical Biochemistry* 246:20, 1997).

In situ assays produce a visible activity that co-localizes with ligand-receptor binding, revealing both binding and the bound ligand's reagent history simultaneously. In some embodiments, in situ assays take place in one or more polymeric films overlaid on the support. The polymeric films contain one or more receptors and/or indicator compounds in a polymer matrix comprising, for example, agarose, polyacrylamide, polyvinyl alcohol, polyvinyl alcohol modified with stilbazolium groups, or any other such polymer compatible with detecting binding of particular ligands and receptors. In some embodiments, the films will be photopatternable, and will typically swell when hydrated forming a polymeric gel. After either chemical or photolytic release from the support, ligands will diffuse into the surrounding gel matrix. If a particular group of ligands specifically binds the receptors in the gel, then a zone of activity will be visible around that group. Determining the position of the element will reveal the reagent history, or more preferably, the composition of the ligand in a straightforward fashion. In the case of a porous array, the position is readily determined by the landmark features of the array where individual ligand groups correspond to discrete porous layers.

In other embodiments, the surface between array elements is modified with an organosilane providing a differential surface tension between the surface and the individual array elements (see You et al., *Chemistry & Biology* 4:969, 1997). The surface tension causes an applied receptor solution to segregate into individual droplets, with each droplet adhering to a separate array element. Exposure to either light or chemicals releases the ligands into the droplet, which in preferred embodiments is a nanodroplet (i.e., on the order of 10^{-9} liter). The spatial segregation of droplets prevents the mixing of detached ligands from other array elements. As a result, each array element is assayed for ligand-receptor binding in the liquid-phase using an in situ assay mixture. One advantage of screening detached ligands directly in solution is that it avoids potential complications of a polymeric film. As such, it is potentially a more generally applicable method.

In another embodiment, screening for ligand-receptor binding may be performed in vivo using living cells in direct contact with the array surface. According to this embodiment, linkers are provided with photolabile or enzyme-cleavable groups, which enables removal of ligands by contact with elements that are compatible with living cells. The enzyme-cleavable group is preferably chosen so as to be substantially cleavable with enzymes secreted by living cells. Most preferably, the cell will secrete an enzyme that detaches the ligand from the array, permitting the ligand to subsequently diffuse into the cell and affect an internal biologic process (i.e., ligand-receptor binding occurs in vivo). For example, arrays of nucleobase polymers attached via protease-sensitive linkages may be used to conduct antisense experiments on cells growing in direct contact with the surface of the array. Ligand separation from the support is essential for transmigration of the ligand through the cell membrane. Cell-induced cleavage of the ligand also allows the separated ligands to remain co-localized with their site of attachment, and the cells in contact with that site. Co-localization is particularly advantageous when a phenotypic cellular assay is used to determine modulation of gene expression by a nucleobase polymer. In such an assay, determining the location of the phenotypic change determines the sequence of the nucleobase polymer affecting the change, as well as the base sequence of its putative intracellular target. By using articles comprising many thousands

of unique nucleobase polymers, such an approach is extremely powerful in that a single experiment can potentially determine the effect of every single gene in an entire genome on a particular phenotype.

As noted above, certain ligands synthesized by the methods described herein may comprise a target receptor modifying group that detectably alters a bound receptor. Ligands that comprise such a modifying group may be used within methods for modifying a target receptor. Such methods comprise contacting an array comprising ligands that contain such a group with a target receptor, which may be isolated or present within a mixture. It will be apparent that such contact should be performed under conditions and for a time sufficient to permit the desired modification.

Alternatively, ligands may be used as reagents in chemical or enzymatic reactions, rather than only being the subject of analysis as described above. In some embodiments, the increased ligand surface densities of porous arrays will provide sufficient material to perform arrays of meaningful enzymatic reactions. For example, single nucleotide differences may be detected by polymerase extension of oligonucleotides arrayed on the porous support (see Nikiforov et al., *Nucleic Acids Res.* 22:4167, 1994; Shumaker et al., *Human Mutation* 7:346, 1996; Pastinen et al., *Genome Research* 7:606, 1997 and Lockley et al., *Nucleic Acids Res.* 25:1313, 1997). Alternatively, arrays of primer pairs may be used to conduct arrays of amplification reactions using, for example, PCR. In some embodiments, such enzymatic reactions might occur in situ in one or more polymeric films overlaid on the porous coating. Alternatively, enzymatic reactions may be performed separately by removing array elements to individual reaction vessels.

Still further applications of the invention include information storage, production of molecular-electronic devices, production of a stationary phase in microfabricated separation devices, photography, and immobilization of labeled and unlabeled cells, proteins, antibodies, lectins, nucleic acids, nucleic acid probes, polysaccharides and the like in a pattern on a surface.

In yet another embodiment, arrays provided herein may be used in preparative applications wherein ligand arrays attached to a substrate are used to isolate a complementary target receptor from a mixture of receptors using methods analogous to those above for screening for receptor binding. Within such methods, a composition comprising a target receptor is contacted with a ligand-array as provided herein, provided that at least one nucleobase attached to the array binds to the target receptor. Unbound components of the composition are then removed from the array. The target receptor may then be separated from the array by altering conditions such that ligand-receptor binding is diminished.

In other embodiments, ligands or bound receptors may be selectively isolated from the array using a photoresist layer as described in U.S. Pat. No. 6,159,681 entitled "Light-Mediated Method and Apparatus For the Regional Analysis of Biologic Material". Briefly, by establishing a photoresist layer over ligands of the array and/or bound receptors, it is possible to precisely irradiate regions of the photoresist to expose specific ligands and/or receptors. Exposed ligands or receptors may then be selectively isolated by detaching them according to methods described above. Once isolated, the detached material may be further analyzed using any of a variety of analytic methods.

There are a variety of assays, including diagnostic assays, that involve the hybridization of an antisense molecule to a target nucleic acid molecule, either isolated or present within a mixture of compounds. Arrays as provided herein may be used within such hybridization steps. Such arrays should contain attached antisense molecules (i.e., nucleobase polymers that specifically and detectably bind to nucleic acid molecules of complementary sequence under moderately stringent conditions). Ligands may, but need not, be detached from the surface either before or after hybridization. In general, such hybridization reactions should be performed under conditions that favor specific hybridization. Suitable conditions may be selected by those of ordinary skill in the art.

The following Examples are offered by way of illustration and not by way of limitation. Within these Examples, all operations were conducted at about ambient temperatures and pressures unless indicated to the contrary.

EXAMPLES

Example 1

Attachment of an Adhesive Layer

This Example describes the attachment of an adhesive layer to a glass substrate, prior to application of a porous coating.

An adhesive layer was prepared on a commercial glass microscope slide (Curtin-Matheson Scientific, Inc., Houston, Tex.). The adhesive layer was prepared from a clear sol obtained by hydrolyzing and aging tetraethoxysilane (Aldrich Chemical Company, Inc., Milwaukee, Wis.). To prepare the sol, a concentrated sol was first prepared by hydrolyzing 21.7 ml of tetraethoxysilane in 6.3 ml H_2O and 0.7 ml IN nitric acid at room temperature for approximately 1 hour, followed by aging at 4° C. for several days. The concentrated sol was diluted 50-fold with ethanol, and applied to one surface of the slide at an incline using a pipette. The solvent was allowed to evaporate at room temperature leaving an adhesive layer less than 1 μm thick, and a free adhesive surface. The layer was cured by placing the slide on a heating block at 110° C. to 120° C. for 15 minutes.

Examples 2–8

Preparation of Representative Porous Coatings

These Examples describe the preparation of representative porous coatings.

The porous coating was obtained from a liquid coating solution. The coating solutions were prepared as follows: Five grams of fumed silica (SiO_2) with a primary particle size of 500 Å (Degussa, Inc., Ridgefield Park, NJ) were dispersed in 100 ml of 95% ethanol (5% water). To this dispersion was added tetraethoxysilane (TEOS) monomer or polymer in an amount indicated in Table VIII. TEOS polymer was obtained as described in Example 1. Sufficient nitric acid was added to provide an acidity of from 2.0 to 4.2 pH units. Coating solutions that incorporated linker molecules further included 3-aminopropyltriethoxysilane (i.e., "APES", from Aldrich Chemical Company, Inc., Milwaukee, Wis.) or bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane (i.e., "HAPES", from Gelest, Inc., Tullytown, Pa.) in amounts as indicated in Table VIII. The coating solutions thus formed were stirred in a plastic container at room temperature for greater than 24 hours. Subsequent to aging, the coating solutions were applied to an adhesive surface of glass slides prepared as described in Example 1. The coating solutions were applied at an incline using a Pasteur pipette producing substantially uniform liquid layers. The liquid layers were allowed to evaporate at room temperature leaving a series of continuous porous coatings from 1 μm to 4 μm thick. The layers were then cured by heating to 120° C. for 15 minutes. The series of porous coatings were evaluated with respect to film quality as summarized in Table VIII.

TABLE VII

Example Number	TEOS, μmole/g silica	TEOS polymerized	Linker, μmole/g silica	pH	Age of Mix	Majority Solvent	Film Quality
2	372	yes	—	3.6	>24 hrs	3-pentanone	uniform
3	1860	yes	—	2.8	>24 hrs	3-pentanone	uniform[1]
4	1860	no	—	2.8	>24 hrs	ethanol	uniform[2]
5	240	no	—	4.2	>24 hrs	ethanol	uniform
6	80	no	HAPES, 160	4.0	>24 hrs	ethanol	uniform
7	180	yes	APES, 60	2.0	>24 hrs	ethanol	uniform
8	180	no	APES, 60	4.2	66 hrs	ethanol	uniform

[1,2]Greater than 25 μm thick.

Table VIII illustrates that uniform porous coatings were obtained from coating solutions prepared by contacting silica particles with either hydrolyzed metal alkoxide (Examples 4 and 5), or with metal alkoxide polymers formed separately (Examples 2 and 3). Uniform porous coatings greater than 25 μm thick were formed from coating solutions containing different solvents as Examples 3 and 4 demonstrate with 3-pentanone and ethanol, respectively. Table VIII also illustrates that it is possible to obtain uniform porous coatings with attached linker molecules by incorporating linker molecules directly in the coating solution as in Examples 6, 7, and 8. Such coating solutions are sensitive to how the TEOS and linker copolymers are formed. As illustrated in Examples 7 and 8, the coating solution obtained by adding APES to TEOS polymers has a lower pH optimum for uniform film formation than the coating solution obtained by adding APES to TEOS monomers.

Examples 9–18

Comparative Coatings

These Examples illustrate the preparation of coatings in a manner similar to those in Examples 2–8, with modifications as summarized in Table IX. The coating solutions were applied to the adhesive surface of glass slides prepared as in Example 1, producing substantially uniform liquid layers. The liquid layers were allowed to evaporate at room temperature followed by curing at 120° C. for 15 minutes. The series of coatings were evaluated with respect to film quality as reported in Table IX.

TABLE IX

Example Number	TEOS, μmole/g silica	TEOS polymerized	Linker, μmole/g silica	pH	Age of Mix	Majority Solvent	Film Quality
9	—	—	—	—	NA	ethanol	separated
10	—	—	—	—	NA	water	cracked
11	—	—	—	—	NA	3-pentanone	separated
12	1860	no	—	2.8	<1 hr	3-pentanone	flocculated
13	744	yes	—	4.2	<1 hr	ethanol	separated
14	240	no	—	>10	>24 hrs	ethanol	clumped
15	—	—	APES, 60	4.1	>24 hrs	ethanol	flocculated
16	180	no	HAPES, 60	4.0	>24 hrs	ethanol	clumped
17	180	yes	APES, 60	4.1	>24 hrs	ethanol	clumped
18	180	no	APES, 60	4.2	18 hrs	ethanol	separated

[1]NA - not applicable.

All the Examples in Table IX yield coatings of inferior film quality compared to the Examples in Table VIII. Examples 9–11 produced coatings that were cracked or separated at a macroscopic and microscopic level, demonstrating that a polymeric component, such as TEOS, is necessary to form uniform porous coatings with fumed silica particles. Examples 12, 13 and 18 demonstrate that insufficient aging of this coating solution leads to flocculated and separated porous coatings (compare with Examples 2, 3, 4, and 8). Insufficient aging presumably prevents extended polymer formation (Examples 12 and 18) and/or the attachment of extended polymers to silica particles (Example 13). Example 14 illustrates that even with sufficient aging, increasing the pH above about 7.0 pH units results in solution instability due to particle aggregation (compare with Example 5). Organoalkoxysilanes containing an amino moiety such as APES do not function as substitutes for TEOS, as shown in Example 15. It is recognized that APES forms less extended polymers than TEOS, possibly explaining its ineffectiveness. As illustrated, coating solutions made by the random copolymerization of TEOS and a linker molecule are sensitive to the order of polymerization (compare Examples 8 and 17), the relative stoichiometry of TEOS and linker molecule (compare Examples 6 and 16), and the pH of the mixture (compare Examples 7 and 17).

Example 19

Preparation of a Photopatterned and Fortified Porous Coating

This Example illustrates the patterning of a porous coating, and the application of a fortifying layer to a patterned porous coating.

A coating solution was prepared according to the procedure described in Example 5 above. The coating solution was applied to the adhesive surface of a glass slide prepared as in Example 1, producing a substantially uniform liquid layer. The liquid layer was allowed to evaporate at room

temperature leaving a uniform porous coating. Curing was postponed until after photopatterning.

While working in a laminar flow hood illuminated by cool-white fluorescent lights shielded with Gold Shields™ (Imtec Products Inc., Sunnyvale, Calif.), AZ® 1512 positive photoresist (Hoechst Celanese™, Somerville, NJ) with a solids content of 26 weight percent was diluted three-fold with propylene glycol methyl ether acetate (PGMEA) and applied to the porous coating using a Pasteur pipette. The excess was allowed to drain onto a paper towel by positioning the slide vertically. The slide was then placed on a flat surface for approximately 10 minutes at room temperature to substantially evaporate the solvent, followed by soft-baking on a metal heating block at a temperature of from 90° C. to 100° C. for 10 to 15 seconds. The evaporated layer of photoresist substantially covered the porous coating.

The photoresist surface was brought into contact with a mask bearing a 16×16 array of 600 μm×600 μm opaque squares on a transparent background (Precision Image Corporation, Redmond, Wash.). The opaque squares were separated from one another by 200 μm. The mask was exposed to 365 nm light at an energy density of 8 mW/cm^2 for 90 seconds using a UV transilluminator (UVP Inc., Upland, Calif.).

With the photoresist appropriately irradiated, the entire substrate was immersed in AZ® 351 developer diluted six-fold with distilled water (Hoechst Celanese™, Somerville, N.J.). The photoresist in irradiated regions and the porous coating within it were both completely removed from the substrate surface after about 60 to 120 seconds in developer. The temporal progress of dissolution was visually monitored by the formation of red dye from irradiated regions during the development process. After development, the entire substrate was rinsed with distilled water, and allowed to air-dry. Unirradiated photoresist was stripped by immersion in acetone followed by an acetone rinse and evaporation. Stripping of unirradiated photoresist left a patterned porous coating comprising a 16×16 array of porous squares.

FIG. **2** is a scanning electron microscope print of the patterned porous coating at a magnification of 1,100×, showing a corner of one porous square. As shown, the porous square has a continuous porous coating (i.e., the coating covers the surface of the substrate with virtually no discontinuities or gaps), which is uniformly 2 μm thick. FIG. **3** is a scanning electron microscope print of the porous coating in FIG. **2** at a magnification of 95,000×. At very high magnification, the porous coating is shown to be comprised of a rigid and continuous network of silica particles with an extensive surface area wherein the average pore size approximates the primary particle size.

A fortifying solution was applied as a separate coating after photopatterning to further anchor the elements of the porous coating without substantially filling the pore volume. The fortifying solution was a 150-fold ethanol dilution of the concentrated sol prepared in Example 1. The fortifying solution was applied to the patterned porous coating at an incline using a pipette. The solvent was allowed to evaporate at room temperature followed by curing at 110° C. to 120° C. for 15 minutes.

This Example demonstrates that the porous coatings of the present invention can be (1) tailored to have a particular average pore size by choosing an appropriate primary particle size, (2) photopatterned with high sensitivity (AZ® 1512 positive photoresist has a photospeed of 58 mJ/cm^2) and (3) photopatterned using microfabrication techniques.

Example 20

Attachment of Linker Molecules

This Example illustrates the attachment of linkers to a patterned porous coating prepared as described in Example 19.

The coating was immersed in AZ® 351 developer diluted six-fold with distilled water for 15 seconds, and rinsed with distilled water. Linker molecules were coupled to the coating surface by immersing the coating in a 2% solution of an organoalkoxysilane in ethanol-H_2O (95:5) for 10 minutes, followed by rinsing with ethanol and curing at 120° C. for 15 minutes. The coating was again immersed in AZ® 351 developer for 15 seconds, rinsed with distilled water, and dried. The basic aqueous developer deprotinates surface silanol and amino groups, facilitating subsequent process steps. Patterned porous coatings with attached amino-linker molecules (i.e., amino reactive group) and hydroxyl-linker molecules (i.e., hydroxyl reactive group) were obtained by using APES and HAPES, respectively, as the organoalkoxysilane.

Example 21

Ligand Surface Density: Comparative Example

This Example illustrates, for comparative purposes, ligand surface density on a substrate having a porous coating, as compared to the density on a substrate lacking a porous coating.

A substrate lacking a porous coating was coupled with amino-linker molecules using the method described in Example 20. Using an appropriately patterned photoresist layer applied to the surface, the amino-linker molecules were reacted with fluorescein isothiocyanate (FITC) in a specific pattern comprising a 16×16 array of 600 μm×600 μm squares. This FITC-labeled slide served to demonstrate the ligand surface density provided by the prior art (for representative reference, see Fodor et al., *Science* 251:767, 1991).

While working under light greater than 500 nm, PVA-SBQ photoresist was applied to the linker-modified slide surface as a thin liquid layer. The liquid layer was baked on a metal heating block at a temperature of from 90° C. to 100° C. for 15 seconds leaving a 1–2 μm thick photoresist film. PVA-SBQ photoresist is a previously described negative photoresist derived from stilbazolium (SBQ) substituted polyvinyl alcohol (PVA) (see Ichimura et al., U.S. Pat. No. 4,891,300). Irradiated regions of the photoresist undergo a light-induced cross-linking of SBQ groups rendering those regions insoluble in an aqueous developer. PVA-SBQ photoresist was chosen as the photopatternable barrier layer because the light-induced cross-linking of SBQ groups is chemically inert with regard to the underlying amino-linker molecules. The PVA-SBQ composition used to coat the linker-modified slide comprised 0.8% PVA-SBQ and 0.025% Triton X-100 in 70% ethanol and 30% water.

The surface of the photoresist film was brought into contact with a mask bearing a 16×16 array of 600 μm×600 μm opaque squares on a transparent background. The opaque squares are separated from one another by 200 μm. The mask was exposed to 365 nm light at an energy of 8 mW/cm^2 for 7 seconds using a UV transilluminator (UVP Inc., Upland, Calif.).

With the photoresist appropriately irradiated, the entire substrate was immersed in distilled water which dissolved unirradiated regions of the PVA-SBQ photoresist leaving a

cross-linked photoresist layer with a 16×16 array of square openings to the underlying substrate surface and the attached amino-linker molecules. The remaining surface was blocked by a barrier layer comprising the cross-linked photoresist layer.

The FITC coupling reaction was performed by immersing the slide in 0.1 mM FITC in acetonitrile for 5 minutes at room temperature followed by acetonitrile and acetone washes. Because of the patterned barrier layer on the surface of the slide, FITC only coupled to the surface in a pattern corresponding to the 16×16 array of square openings. After FITC coupling, the cross-linked photoresist layer was stripped by immersing the slide in a 0.75% aqueous solution of sodium periodate ($NaIO_4$) for 30 seconds (i.e. solubilizes the film by cleaving 1,2-diol units in the PVA). The slide was then washed with water and dried.

The fluorescence image of the FITC-labeled slide was captured at three different magnifications using a 35 mm camera attached to a Standard Epifluorescence Microscope (Carl Zeiss, Thornwood, N.Y.). Using an exposure time of 15 seconds, the fluorescence image of the square array was faint at objective magnifications of 10× and 20×, and barely visible at an objective magnification of 2× (see FIGS. **4**A, **4**C and **4**E).

A patterned porous coating was prepared as described ill Example 19, and coupled with amino-linker molecules using the method described in Example 20. The amino-linker molecules of the porous coating were reacted with fluorescein isothiocyanate (FITC) which forms a covalent linkage with the amino group of the linker molecules. This FITC-labeled porous coating was utilized to demonstrate the increase in ligand surface density provided by the present invention.

The FITC coupling reaction was performed by immersing the linker-modified porous coating in 0.1 mM FITC in acetonitrile for 5 minutes at room temperature followed by acetonitrile and acetone washes. The fluorescence image of the FITC-labeled porous coating was captured at three different magnifications using a 35 mm camera attached to a Standard Epifluorescence Microscope. Using an exposure time of 15 seconds, the fluorescence image of the patterned porous coating is readily visible using objective magnifications of 2×, 10×, and 20× (see FIGS. **4**B, **4**D and **4**F).

This example demonstrates that (1) compared with the image intensity of the prior art, the present invention provides a marked increase in ligand surface density, (2) imaging of ligands on the patterned porous coating is rapid and economical using standard equipment, (3) the patterned porous coating is compatible with methods of solid-phase synthesis, and (4) the porous coating does not swell or distort during solid-phase synthesis.

Example 22

Specific Binding of a DNA-ligand by a DNA-receptor

DNA-ligands were synthesized on the surface of a patterned porous coating using solid-phase synthesis in order to demonstrate that a DNA-receptor specifically binds to a complementary surface-bound DNA-ligand. A patterned porous coating was prepared as described in Example 19, and coupled with hydroxyl-linker molecules using the method described in Example 20.

A reactor system was formed by mating the slide to a polytetrafluoroethylene (i.e., PTFE, marketed as TEFLON®) reactor base with an intervening PTFE gasket. Sandwiched together, the slide, gasket, and base formed a sealed reactor cavity except for inlet and outlet ports in the reactor base as shown in FIG. **1**E. The reactor cavity had a volume of 300 μl and the patterned porous coating was fully contained with the cavity. The reactor system allowed chemical reagents to be delivered over the patterned porous coating either manually or automatically by connecting the inlet and outlet ports to either syringes or a reagent delivery machine, respectively. In this example, the inlet and outlet ports were connected to a PCR-Mate® automated DNA synthesizer (Applied Biosystems, Inc., Foster City, Calif.).

Using the DNA synthesizer to automatically deliver reagents, the DNA-ligand with the sequence 5'-GCCTACGC-3' was synthesized via eight phosphoramidite couplings on the exposed hydroxyl groups beginning with the 3' end. Following synthesis, the reactor system was separated from the DNA synthesizer, and the phosphate and exocyclic amine protecting groups removed by manually applying 0.05 M potassium carbonate in methanol to the reactor cavity for 2 hours at room temperature. The strongly basic conditions (i.e., concentrated ammonium hydroxide) ordinarily used to deprotect DNA after solid-phase synthesis were avoided because of the possibility of cleaving the siloxane bonds connecting the linker molecules to the substrate surface. Instead, deprotection was accomplished using the mildly basic conditions described above by using phenoxyacetyl (Pac) protected dA, 4-isopropyl-phenoxyacetyl (iPr-Pac) protected dG, and acetyl protected dC (Glen Research, Inc., Sterling, Va.).

Following deprotection, the slide was separated from the reactor base and visualized using fluorescence microscopy without the application of a labeled receptor. The image was captured using a 35 mm camera attached to a Standard Epifluorescence Microscope (Carl Zeiss, Thornwood, N.Y.). As shown in FIG. **5**, the fluorescence image and corresponding surface plot of the porous coating reveal no detectable background fluorescence.

To demonstrate the availability of surface-bound DNA for hybridization with a complementary DNA-receptor, 20 μl of 10 μM 5'-GCGTAGGC-fluorescein "FTA" in FIGS. **5**C and **5**D) suspended in 6×SSPE was applied to the porous surface and incubated for an hour at a temperature of 4° C. The 6×SSPE solution is a high-salt buffer with a pH of 7.4 comprising 1M NaCl, 66 mM NaH_2PO_4/Na_2HPO_4, 6 mM EDTA, and 0.05° Triton X-100-™ The slide was subsequently washed in 6×SSPE at a temperature of 15° C., and mounted on a modified microscope stage attached to a Standard Epifluorescence Microscope The stage contained an aqueous gel within its interior that was chilled to approximately 4° C. prior to image acquisition. The fluorescence image of FTA hybridized to the porous coating was captured using a 35 mm camera attached to the microscope. Using an exposure time of 15 seconds, the fluorescence image of the hybridized DNA-receptor was readily visible using an objective magnification of 10× (see FIGS. **5**C and **5**D and corresponding surface plot). The bound DNA-receptor was dissociated from the ligand-DNA by immersing the slide in 45° C. water, and the hybridization repeated. This cycle of thermal dissociation and hybridization was repeated at least 20 times with no loss in the fluorescence intensity from bound FTA, demonstrating the stability of the DNA-ligand attachment.

To demonstrate the sequence specificity of DNA hybridization, FTA was thermally dissociated from the porous surface and 20 μl of 10 μM 5'-GCGAAGGC-fluorescein "FAA" in FIGS. **5**E and **5**F; FAA differs from FTA at the underlined base) suspended in 6×SSPE was

applied to the porous surface and incubated for an hour at a temperature of 4° C. Following hybridization, the slide was washed in 6×SSPE at a temperature of 15° C. and mounted on the modified stage as described above. The fluorescence image of the FAA hybridization was captured with the 10× objective of a Standard Epifluorescence Microscope with attached 35 mm camera. The fluorescence image and corresponding surface plot of the FAA hybridization reaction are shown in FIGS. **5**E and **5**F (exposure time of 15 seconds). Although FTA and FAA differ by only a single base, hybridization is highly specific with the images of FAA hybridization equivalent to the images with no receptor.

This example demonstrates that (1) the porous coating provides a successful substrate for performing solid-phase synthesis, (2) the porous coating has low autofluorescence, (3) the porous coating provides an increased DNA-ligand surface density, (4) surface-bound DNA-ligands are available for DNA-receptor binding, (5) imaging of labeled receptors on the patterned porous coating is rapid and economical using standard equipment, and (6) the porous coating provides a substrate capable of detecting the specific binding characteristic of macromolecular receptors.

Example 23

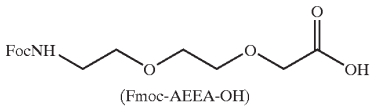
Specific Binding of a PNA-array by a DNA-receptor

This Example illustrates the specific binding of a DNA-receptor to a complementary member of a PNA-array comprising 16 different PNA sequences on the surface of a patterned porous coating.

A PNA array comprising 16 different PNA sequences was synthesized on the surface of a patterned porous coating as described above. The PNA array was synthesized using solid-phase synthesis and methods more fully described in co-pending Application Ser. No. 09/326,479 entitled, "Methods and Compositions for Performing an Array of Chemical Reactions on a Support Surface" and U.S. Pat. No. 6,569,598 entitled "Solvent-Resistant Photosensitive Compositions," incorporated herein by reference. All PNA reagents were from PerSeptive Biosystems, Inc. (Framingham, MA). PNA sequences with protecting groups on the exocyclic amines are indicated by the base designations A, G, C, and T to distinguish them from deprotected sequences which use A, G, C, and T as base designations.

A patterned porous coating comprising a 4×4 array of 600 μm×600 μm porous squares was prepared as described in Example 19, and coupled with amino-linker molecules using the method described in Example 20. The PNA-array was then synthesized on the surface of the patterned porous coating with each array element occupying one 600 μm×600 μm porous square. Each element of the array comprised one of the 16 possible combinations of the sequence, linker-spacer-CGN_1N_2TCCG-NH_2, where N_1 and N_2 may independently be A, G, C, or T. The position of each element in the array, as referenced by the N_1N_2 sequence, is shown in the array schematics of FIGS. **6**A and **6**B. The shaded grid in each schematic indicates the array element that is complementary to the receptor sequence above each schematic. The portions of the DNA-receptors complementary to the corresponding N_1N_2 sequence are underlined.

The PNA-array was synthesized by first attaching the sequence, linker-spacer-CG-NH-Fmoc to all porous squares by manually applying reagents using the reactor system described in Example 22. (Fmoc: fluorenylmethyloxycarbonyl, a base-labile amino-protecting group removed under nonhydrolytic conditions). The spacer is Fmoc-AEEA-OH, a molecule with the following formula:



(Fmoc-AEEA-OH)

The spacer was coupled to the free amino group of the linker molecules by two 120 μl applications of a solution comprising 72 mM Fmoc-AEEA-OH, 60 mM HATU, 100 mM 2,6-lutidine, and 66 mM DIPEA in 33% NMP and 66% DMF. The linker molecules were incubated with the spacer solution for 30 to 40 minutes, followed by a DMF rinse. Unreacted linker molecules were then capped from further reaction by applying a solution comprising 10% acetic anhydride and 10% 2,6-lutidine in tetrahydrofuran for 5 minutes. The reactor cavity was flushed with DMF, and the Fmoc protecting groups removed with 1 ml of 20% piperidine in DMF flowed continuously through the reactor cavity over 10 minutes to drive the reaction to completion. The C monomer was then coupled to the free amino group of the spacer using the coupling conditions described above. Capping and Fmoc removal led to the sequence, linker-spacer-C—NH_2. This sequence was coupled with the G monomer and capped, but not deprotected. This provided the sequence, linker-spacer-CG-NH-Fmoc.

The N_1N_2 sequence was next added using a series of photopatterned barrier layers consisting of the positive photoresist described in U.S. Pat. No. 6,569,598 entitled "Solvent Resistant Photosensitive Compositions." Two main properties of the positive photoresist make it particularly useful as a barrier layer during solid-phase synthesis. First, it is resistant to many organic solvents that are used in organic reactions. Second, irradiated regions of the photoresist undergo a photochemical reaction that is inert with respect to chemical species attached to the underlying surface. Using a series of 8 patterned photoresist layers applied to the surface, two layers of PNA monomers were selectively applied to the patterned porous coating creating one of the 16 possible combinations of the sequence, linker-spacer-CGN_1N_2—NH-Fmoc at each porous square.

In detail, a photoresist layer was established on the porous support bearing the sequence, linker-spacer-CG-NH-Fmoc at all porous squares. The surface of the photoresist film was brought into contact with an opaque mask bearing a transparent rectangle comprising the rectangular region occupied by the first column of four porous squares. The mask was exposed to 365 nm light at an energy density of 8 mW/cm^2 for 10 minutes using a UV transilluminator. With the photoresist appropriately irradiated, the entire substrate was immersed in developer which dissolved the irradiated region leaving a photoresist layer with a rectangular opening to the first column of porous squares. The slide was attached to the reactor base, and the region encompassing all porous squares was exposed to 20% piperidine in toluene as described above. Because of the patterned barrier layer on the surface of the slide however, piperidine removed Fmoc only from the first column of porous squares. The slide was detached from the reactor base, and the photoresist layer stripped with an organic solvent.

The slide was reattached, and the entire array of porous squares contacted with A monomer using the conditions described above. The A monomer only coupled to the first column of porous squares where Fmoc was selectively removed. Following capping, another photoresist layer was

established, and irradiated in a region comprising the second column of porous squares. The second column of porous squares was selectively deprotected and coupled with G monomer as described above. This process was repeated for the third and fourth columns using C and T monomers, respectively.

After the application of these 4 patterned barrier layers, the N_1 layer was completed creating one of the four sequences, linker-spacer-CGN_1—NH-Fmoc, at each column. The N_2 layer was completed similarly, except that the transparent rectangle of each of the 4 masks comprised the region occupied by a row of four porous squares. With the N_2 layer completed, all Fmoc groups were removed and the remaining sequence synthesized by successively coupling T, C, C, and G monomers to all porous squares. The exocyclic amine groups in this example utilized Bhoc (benzhydryloxycarbonyl) protection, which allows short deprotection times. Accordingly, the exocyclic amine protecting groups were removed by a 10 minute incubation with 25% m-cresol in TFA. Deprotection gives the 16 element PNA-array, with each porous square comprising a single element of the sequence, linker-spacer-$CGN_1N_2TCCG-NH_2$. As an example of PNA-array elements expressed in terms of reagent history, the "CT" and "CA" elements in the schematic may be written as follows:

CT: S-[APES]-[Fmoc-AEEA-OH]-[cap]-[pip]-[C]-[cap]-[pip]-[G]-[cap]-[A]-[G]-[pip]-[C]-[T]-[pip]-[T]-[C]-[G]-[A]-[pip]-[T]-[pip]-[C]-[pip]-[C]-[pip]-[G]-[TFA]

CA: S-[APES]-[Fmoc-AEEA-OH]-[cap]-[pip]-[C]-[cap]-[pip]-[G]-[cap]-[A]-[G]-[pip]-[C]-[T]-[T]-[C]-[G]-[pip]-[A]-[pip]-[T]-[pip]-[C]-[pip]-[C]-[pip]-[G]-[TFA]

where "cap" is the capping reagent, "pip" is the Fmoc removal reagent, and T, C, G, and A are Fmoc and Bhoc protected monomer coupling reagents.

To demonstrate the specificity of DNA hybridization to the PNA-array, 10 μM FAA in 6×SSPE was applied to the porous surface and incubated for 10 minutes at room temperature, followed by a brief room temperature wash in 6×SSPE. The fluorescence image of the porous coating was captured at room temperature using a 35 mm camera attached to a Standard Epifluorescence Microscope. The fluorescence image of FAA bound at the predicted array element is readily detected and visualized using an objective magnification of 10× and an exposure time of 15 seconds (see FIG. **6** and corresponding surface plot). Although six of the array elements differ from the "CT" element by only a single base, FAA hybridizes specifically to its complementary PNA sequence with little or no signal from other array elements.

Bound FAA was dissociated from the PNA-array by immersing the slide in 90° C. water, and the hybridization repeated with FTA using conditions as described above for FAA. The fluorescence image of the FTA hybridization was captured with the 10× objective of a Standard Epifluorescence Microscope with attached 35 mm camera. The fluorescence image and corresponding surface plot are shown in FIG. **6** (exposure time of 15 seconds). As with FAA, FTA hybridizes specifically to its complementary PNA sequence with little or no signal from other array elements.

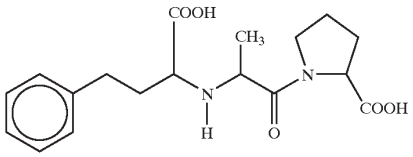
This Example demonstrates that (1) the porous coating provides a successful substrate for performing solid-phase synthesis of ligand arrays, (2) the porous coating provides an increased PNA-ligand surface density, (3) surface-bound PNA-ligands are available for DNA-receptor binding, (4) imaging of labeled receptors on the patterned porous array is rapid and economical using standard equipment, and (5) the porous coating provides a substrate capable of detecting the specific binding characteristic of macromolecular receptors. This Example further demonstrates the advantages of PNA arrays over DNA arrays including more rapid hybridization (10 minutes versus 60 minutes for DNA), greater specificity, and more convenient hybridization conditions (i.e., hybridization of short probes at room temperature).

The Example also illustrates the advantage of synthesizing a PNA array directly on the support used for screening, as opposed to applying an array of presynthesized PNA molecules to a support. While PNA molecules are readily synthesized on a solid-phase, many sequences aggregate after being cleaved from the support. By synthesizing the PNA array directly on the porous support, aggregation and issues of solubility are avoided. As a result, the disclosed PNA arrays do not have the sequence or length restrictions typically encountered with solution-phase hybridization of PNA with DNA.

Example 24

An Array of Weakly Inhibitory Drug Analogues: Synthesis and Screening

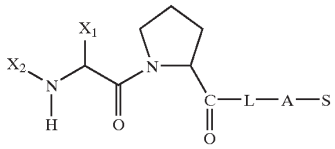
This Example illustrates the preparation of an array comprising nine analogues of enalaprilat, in order to demonstrate the efficacy of the method for synthesizing and screening arrays bearing low-molecular-weight compounds characteristic of drugs, herbicides, and pesticides. Enalaprilat is one of a class of antihypertensives that bind angiotensin-converting enzyme (ACE) and inhibit its dipeptidase activity. ACE generates the powerful vasoconstrictor substance angiotensin II by removing the C-terminal dipeptide from the precursor decapeptide angiotensin I. Enalaprilat is a dipeptide analogue with the following formula:



It is thought that enalaprilat is a transition-state inhibitor with the $CHCO_2H$ and NH groups mimicking the transition state-like geometry attained at the scissile peptide bond of angiotensin I (see Patchett et al., *Science* 288:280, 1980). The enalaprilat array was synthesized using solid-phase synthesis and a series of patterned barrier layers. The reagents were from PerSeptive Biosystems, Inc. (Framingham, MA) except for the α-keto acids which were from Aldrich Chemical Company, Inc., (Milwaukee, Wis.). All amino acids were L-amino acids.

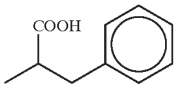
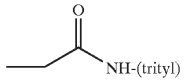
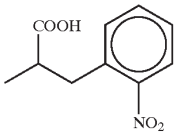
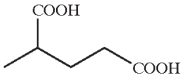
A patterned porous coating comprising a 3×3 array of 1600 μm×1600 μm porous squares was prepared as described in Example 19, and coupled with amino-linker molecules using the method described in Example 20. The enalaprilat array was then synthesized on the surface of the patterned porous coating with each array element occupying one 1600 μm×1600 μm porous square. Each element of the

array comprised one of the nine combinations of compounds with the following formula:

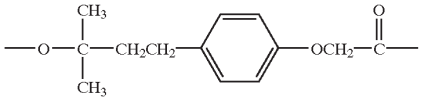


where X_1 may be X_{1a}, X_{1b}, or X_{1c}, and X_2 may be X_{2a}, X_{2b}, or X_{2c} (see Table X).

TABLE X

X_1 Groups	X_2 Groups
X_{1a} = —CH_3	X_{2a} = 
X_{1b} = 	X_{2b} = 
X_{1c} = —O-(tert-butyl)	X_{2c} = 

Within each element, S is the porous surface, A is aminopropyltriethoxysilane, and L is an acid-labile linker with the following formula:



where the tertiary oxygen forms an ester with an enalaprilat analogue, and the carbonyl forms an amide with aminopropyltriethoxysilane. A C-terminal dipeptide may be lost during Fmoc-based solid-phase synthesis through diketopiperazine (DKP) formation (see Gisin and Merrifield, *J. Amer. Chem. Soc.* 94:3102, 1972). The intramolecular aminolysis leading to DKP is particularly accelerated when the C-terminal residue is proline as occurs in the synthesis of enalaprilat analogues. Intramolecular aminolysis and DKP formation were sterically suppressed by connecting proline to the support via an ester of a tertiary alcohol as shown above. The tertiary alcohol was 4-(1',1'-dimethyl-1'-hydroxypropyl)phenoxyacetyl (DHPP; a kind gift from Jan Kochansky, USDA (Beltsville, MD)) as described by Akaji et al., *J. Chem. Soc., Chem. Commun.* 584, 1990 and Kochansky and Wagner, *Tetrahedron Lett.* 33:8007, 1992).

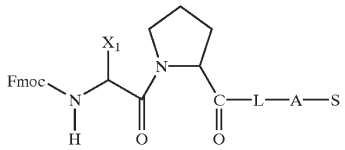
The analogue array was synthesized by first attaching the sequence, S-A-L-Pro-Fmoc to all porous squares by manually applying reagents using the reactor system described in Example 5. DHPP was coupled to the free amino group of A by applying a solution comprising 104 mM DHPP, 93 mM HOAt (1-hydroxy-7-azabenzotriazole), and 105 mM DIPCDI (N,N-diisopropylcarbodiimide) in DMF. Coupling was performed by applying three 100 μl aliquots of the above solution to the reactor cavity over the course of 180 minutes. The reactor cavity was then flushed with DMF, and the tertiary alcohol group of DHPP esterified with 100 mM FMOC-Pro-Cl in pyridine-dichloroethane (1:4) for 20 hours. FMOC-Pro-Cl is the acid chloride of FMOC-Pro prepared from thionyl chloride according to previously published methods (see Carpino et al., *J. Org. Chem.* 51:3732, 1986). The low reactivity of the tertiary alcohol group requires an acid chloride for efficient coupling of Fmoc-Pro to DHPP.

The X_1 and X_2 groups were next added using a series of photopatterned barrier layers consisting of the positive photoresist described in U.S. Pat. No. 6,569,598 entitled "Solvent-Resistant Photosensitive Compositions." The positive photoresist is particularly useful as a barrier layer during solid-phase synthesis because it is resistant to many organic solvents, and irradiated regions of the photoresist undergo a photochemical reaction that is inert with respect to chemical species attached to the underlying surface. Using a series of 6 patterned photoresist layers applied to the surface, two layers of chemical reagents were selectively applied to the patterned porous coating creating one of the above enalaprilat analogues at each porous square. Using a series of 6 patterned photoresist layers applied to the surface, two layers of chemical reagents were selectively applied to the patterned porous coating creating one of the above enalaprilat analogues at each porous square.

In detail, a photoresist layer was established on the porous support bearing the sequence, S-A-L-Pro-Fmoc at all porous squares. The surface of the photoresist film was brought into contact with an opaque mask bearing a transparent rectangle comprising the rectangular region occupied by the first column of three porous squares. The mask was exposed to 365 nm light at an energy density of 8 mW/cm^2 for 10 minutes using a UV transilluminator. With the photoresist appropriately irradiated, the entire substrate was immersed in developer which dissolved the irradiated region leaving a photoresist layer with a rectangular opening to the first column of porous squares. The slide was attached to the reactor base, and the region encompassing all porous squares was exposed to 20% piperidine in toluene as described above. Because of the patterned barrier layer on the surface of the slide however, piperidine removed Fmoc only from the first column of porous squares. The slide was detached from the reactor base, and the photoresist layer stripped with an organic solvent.

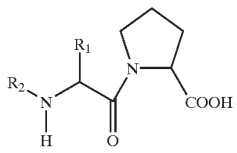
The slide was reattached, and the entire array of porous squares contacted with a solution comprising 233 mM Fmoc-Alanine-OH, 233 mM HATU, and 458 mM DIPEA in DMF. The solution was incubated with the porous surface for 60 minutes, followed by two DMF flushes of the reactor cavity. The Fmoc-Alanine-OH monomer only coupled to the first column of porous squares where Fmoc was selectively removed. Following coupling, another photoresist layer was established, and irradiated in a region comprising the second column of porous squares. The second column of porous squares was selectively deprotected and coupled with Fmoc-Asparagine(trityl)-OH as described above (i.e., trityl protected monomer). This process was repeated for the third column using Fmoc-Serine(tert-butyl)-OH (i.e., tert-butyl protected monomer).

After the application of these patterned barrier layers, the X_1 layer was completed creating one of three compounds at each column with the following formula:



The X_2 layer was attached using similar barrier layers, except that the transparent rectangle of each of the next 3 masks comprised the region occupied by a row of three porous squares rather than a column. The X_2 layer was formed by reductive alkylation of the deprotected amino group with an α-keto acid selected from the group consisting of phenylpyruvic acid, 2-nitrophenylpyruvic acid, and 2-ketoglutaric acid. Each X_2 coupling comprised contacting the entire array of porous squares with a solution of 250 mM α-keto acid and 400 mM $NaBH_3CN$ in acetic acid-DMF (1:99) for 24 hours.

With the X_2 layer completed, a polymeric binder was added to the porous array by applying and evaporating a thin liquid layer of 1% polyvinyl alcohol in ethanol-H_2O (1:3). Each of the porous squares was then removed from the substrate using a razor blade and placed in separate tubes. The polymeric binder prevented fragmentation of the porous network during removal. To each tube was added 200 μl of H_2O followed by centrifugation. The supernatant contained solubilized polymeric binder and was discarded. To each pellet of porous material was added 20 μl of TFA-H_2O (95:5) to cleave the analogues from the support, and remove tert-butyl and trityl protecting groups. After 2 hours of incubation, the TFA-H_2O was removed under vacuum leaving a residue in each tube that contained one of nine enalaprilat analogues with the following general formula:



where R_1 and R_2 are the groups defined in Table XI. Each analogue was then dissolved in 50 μl of 50 mM Tris buffer (pH 8.3) containing 300 mM NaCl. As an example of an analogue expressed in terms of reagent history, the "R_{1a}+R_{2a}"analogue from the array may be written as follows:

R_{1a}+R_{2a}: S-[APES]-[DHPP]-[FMOC-Pro-Cl]-[pip]-[Fmoc-Alanine-OH]-[Fmoc-Asparagine(trityl)OH]-[Fmoc-Serine(tert-butyl)-OH]-[pip]-[phenylpyruvic acid+$NaBH_3CN$]-[2-nitrophenylpyruvic acid+$NaBH_3CN$]-[2-ketoglutaric acid+$NaBH_3CN$]-[PVA]-[H_2O]-[TFA-H_2O]

TABLE XI

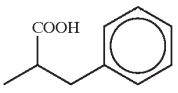
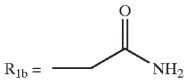
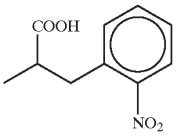
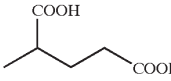
R_1 Groups	R_2 Groups
R_{1a} = —CH_3	R_{2a} = 

TABLE XI-continued

R_1 Groups	R_2 Groups
R_{1b} = 	R_{2b} = 
R_{1c} = —OH	R_{2c} = 

Each enalaprilat analogue was screened for ACE inhibitory activity in a functional assay using the substrate furylacryloylphenyalanylglycylglycine (i.e., FAPGG, Sigma Chemical Co., St. Louis, Mo.). Hydrolysis of FAPGG by ACE results in a decrease in absorbance at 328 nm which can be used to calculate initial enzyme velocities in the presence and absence of enalaprilat analogues. Each assay mixture contained 10 nM ACE, 50 μM FAPGG, 50 mM Tris (pH 8.3), 300 mM NaCl, and 20–40 μl of each of the above analogue solutions in a total reaction volume of 50 μl. The above mixture without analogue served as the negative control for inhibitory activity. A positive control was made by adding 250 nM of the potent inhibitor, lisinopril (IC_{50}= 1.2 nM). Each reaction was initiated by adding 5 μl of 500 μM FAPGG to 45 μl of the remaining assay components in a 100 μl cuvette. The temporal progress of each reaction was monitored by measuring the absorbance at 328 nm every 15 seconds. The negative control had an average initial velocity of 1220 min^{-1}, which compares favorably with the K_m and k_{cat} values reported previously for FAPGG (see Holmquist et al., *Analytical Biochem* 95:540, 1979). The positive control had zero initial velocity.

The percent ACE inhibition of each analogue according to its position in the array is shown in the surface plot of FIG. **7**B. The percent ACE inhibition is expressed as the percent decrease in initial velocity relative to the initial velocity of the negative control. The composition of each enalaprilat analogue in the surface plot may be determined using the array schematic of FIG. **7**A. Compositions are referenced by R_1 and R_2 groups as defined in Table XI. The shaded grid indicates the array element with the highest percent ACE inhibition. The R_{1a}+R_{2a} compound has only moderate binding affinity (IC_{50}=39 nM) compared to enalaprilat (IC_{50}=4.5 nM) as reported by Patchett et al., *Science* 288:280, 1980). Despite its moderate binding affinity, the porous coating of the present invention provided sufficient ligand surface density to detect inhibition of ACE by this compound.

The ligand surface density was calculated for the R_{1a}+R_{2a} compound using the known IC_{50} and the percent inhibition shown in FIG. **7**B. Based on 35 percent inhibition from a 1 μm thick porous coat, the R_{1a}+R_{2a} compound had a calculated surface density of 1.0×10^{-17} mole/μm^2. This is a minimum value since the calculation does not account for losses due to inefficient coupling, deprotection, or cleavage. Even with this caveat, the value is in agreement with the expected range of 0.2×10^{-17} mole/μm^2 to 4.6×10^{-17} mole/uM^2 predicted from reported HAPES and APES surface densities (see Chee et al., *Science* 274:610, 1996 and Fodor et al., U.S. Patent No. 5,510,210). This is equivalent to a ligand concentration in the porous coating of from 0.002 M to 0.040 M. Compare this with the ligand concentration in a polymeric support (e.g., Tenta gel™, RAPP Polymere, GmbH) of from 0.01 M to 0.13 M.

The assay of ACE inhibition by the other analogues both corroborated known structure-function relationships and identified new relationships. For example, it is known that hydrophobic and basic substituents incorporated at R_1 and R_2 result in highly inhibitory compounds (see Patchett et al., *Science* 288:280, 1980). As shown in FIGS. **7**A and **7**B, a nonhydrophic group at R_1 (i.e., R_{1b} or R_{1c}) can have a deleterious effect on inhibitory activity even in the presence of a hydrophobic group at R_2 (i.e., R_{2a}). A negatively charged group at R_2 (i.e., R_{2c}) abolishes inhibitory activity by providing an energetically unfavorable interaction with a putative carboxyl group on the enzyme (compare R_{1a}+R_{2a} and R_{1a}+R_{2c}) Such a carboxyl group would be expected in the enzyme pocket that interacts favorably with inhibitors bearing basic substituents in the R_2 position. Comparing the inhibitory activity of compounds R_{1a}+R_{2a} and R_{1a}+R_{2b} indicates that the 2-nitro group is a moderately unfavorable modification revealing a more subtle structure-function relationship of the active site not previously appreciated. The intermediate effect of the 2-nitro group probably reflects a steric restriction on 2-phenyl substitutions. Despite the low binding affinities of these analogues, the porous coating of the present invention provided sufficient amounts of each compound to identify the above structure-function relationships.

This example demonstrates that (1) the porous coating provides a successful substrate for creating arrays of small-molecule drug candidates using solid-phase synthesis, (2) the ligand surface density is sufficient to perform functional assays using ligands from individual array elements, (3) the ligand surface density is sufficient to perform functional assays using ligands with low to moderate binding affinities, and (5) the porous array of small molecules provides a successful system for identifying relationships between drug structure and drug binding.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except by the appended claims.

What is claimed is:

1. A coated article comprising a substrate having a continuous porous coating of substantially uniform thickness, wherein the coating comprises a gelled network of particles, wherein the porous coating has two or more different compounds attached thereto, and wherein the compounds are attached at known discrete full thickness volumes, each occupying an area on the substrate of less than 1,000.000 μm^2.

2. A coated article according to claim **1**, wherein each particle comprises one or more materials independently selected from the group consisting of carbon, activated carbon, fluorinated carbon, styrenedivinylbenzene copolymers, polystyrene, zeolites, oxides of antimony and oxides of metals present within Group III and Group IV of the Periodic Table.

3. A coated article according to claim **2**, wherein each particle comprises one or more materials independently selected from the group consisting of alumina, silica, silicalite, fumed silica, oxides of tin and titania.

4. A coated article according to claim **1**, wherein the particles are substantially spherical particles of silica.

5. A coated article according to claim **1**, wherein the particles have a primary particle size of less than 1000 Å.

6. A coated article according to claim **1**, wherein the particles have a primary particle size of less than 500 Å.

7. A coated article according to claim **1**, wherein the particles have a primary particle size of less than 100 Å.

8. A coated article according to claim **1**, wherein the particles have a primary particle size of less than 5 Å.

9. A coated article according to claim **1**, wherein the porous coating has a thickness ranging from 0.05 to 25 microns.

10. A coated article according to claim **1**, wherein the porous coating has a surface area that is greater than 50 $meters^2/g$.

11. A coated article according to claim **1**, wherein the porous coating has a surface area of at least 100 square microns per cubic micron of porous coating.

12. A coated article according to claim **1**, wherein the gelled network of particles further comprises a polymer of a hydrolyzed metal alkoxide.

13. A coated article according to claim **12**, wherein the hydrolyzed metal alkoxide has the formula:

$$M(OR)_x$$

wherein M is selected from the group consisting of Si, Ti, Al, B, Zr, Er, Cr, Ga, Ge, Hf, Fe, Ca, Cr, La, Mg, Nb, K, Pr, Sm, Na, Ta, Te, Tl, Sn, W, V, Y, and Zn; R is hydrogen, an alkyl group or an aryl group, and x is 3 or 4.

14. A coated article according to claim **13**, wherein the hydrolyzed metal alkoxide is hydrolyzed tetraethyoxysilane.

15. A coated article according to claim **1**, wherein the substrate is glass.

16. A coated article according to claim **1**, wherein the substrate comprises an adhesive layer in contact with the porous coating.

17. A coated article according to claim **16**, wherein the adhesive layer comprises one or more polymers of a hydrolyzed organo-metal alkoxide of the formula:

$$R_n'M(OR)_x$$

wherein M is selected from the group consisting of Si, Ti, Al, B, Zr, Er, Cr, Ga, Ge, Hf, Fe, Ca, Cr, La, Mg, Nb, K, Pr, Sm, Na, Ta, Te, Tl, Sn, W, V, Y, and Zn; R' is a monovalent organic group containing between 1 and 12 carbon atoms; R is hydrogen, an alkyl group or an aryl group and n and x are integers independently selected from the group consisting of 0, 1, 2, 3 and 4.

18. A coated article according to claim **17**, wherein the adhesive layer comprises a polymer of hydrolyzed tetraethoxysilane.

19. A coated article according to claim **1**, wherein the compounds are covalently attached to the porous coating.

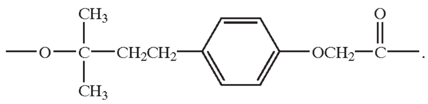
20. A coated article according to claim **1**, wherein the compounds are adsorbed to the porous coating.

21. A coated article according to claim **1**, wherein at least one compound is attached to the porous coating by a linker.

22. A coated article according to claim **21**, wherein the linker comprises a photocleavable moiety or an enzyme cleavable moiety.

23. A coated article according to claim **21**, wherein the linker comprises an acid labile moiety or a base labile moiety.

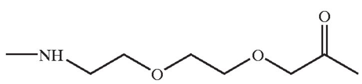
24. A coated article according to claim **23**, wherein the linker comprises the formula:



25. A coated article according to claim **21**, wherein the linker is an organoalkoxysilane molecule attached to the porous coating via a siloxane bond.

26. A coated article according to claim **25**, wherein the linker is 3-amino-propyltriethoxysilane or bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane.

27. A coated article according to claim **21**, wherein the linker comprises the formula:



28. A coated article according to claim **1**, wherein at least 90% of the compounds have the same structure.

29. A coated article according to claim **1**, wherein at least 10% of the compounds have the same structure.

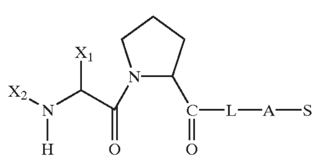
30. A coated article according to claim **1**, wherein at least 10^3 different compounds are attached to the porous coating at known discrete full thickness volumes.

31. A coated article according to claim **1**, wherein at least 10^5 different compounds are attached to the porous coating at known discrete full thickness volumes.

32. A coated article according to claim **1**, wherein at least 10^6 different compounds are attached to the porous coating at known discrete full thickness volumes.

33. A coated article according to claim **1**, wherein the compounds are independently selected from the group consisting of nucleobase polymers and peptides.

34. A coated article according to claim **1**, comprising an enalaprilat analogue of the formula:



wherein S is the porous coating, A is aminopropyltriethoxysilane, L is a divalent linker, X_1 is a monovalent organic group or hydrogen, and X_2 is a monovalent organic group or hydrogen.

35. A coated article according to claim **34**, wherein X_1 is a monovalent organic group comprising one or more acid labile protecting groups.

36. A coated article according to claim **34**, wherein X_2 is a monovalent organic group comprising one or more acid labile protecting groups.

37. A coated article according to claim **1**, wherein the compounds are attached at known discrete full thickness volumes, each occupying an area on the substrate of less than 1,000 μm^2.

38. A coated article according to claim **1**, wherein the compounds are attached at known discrete full thickness volumes, each occupying an area on the substrate of less than 10 μm^2.

39. A coated article according to claim **1**, wherein the average pore size of the porous coating substantially approximates the primary particle size.

40. A coated article according to claim **1**, wherein the average pore size ranges from 1 to 1000 nm.

41. A method for making a coated article with two or more compounds attached thereto, comprising the steps of:

(a) applying to a substrate a substantially uniform layer of a solution comprising metal oxide particles dispersed in a volatile liquid;

(b) evaporating the volatile liquid from the layer, forming a gelled network of metal oxide particles on the substrate, wherein the gelled network forms a porous coating ranging from 0.05 to 25 microns thick; and

(c) attaching two or more compounds to discrete known regions of the porous coating, wherein the compounds are attached at known discrete full thickness volumes, each occupying an area on the substrate of less than 1,000,000 μm^2, and therefrom generating a coated article comprising a substrate having a porous coating with two or more compounds attached thereto.

42. A method according to claim **41**, wherein prior to the step of attaching two or more compounds, the porous coating is cured at a temperature and for a time sufficient to increase the porous coating strength.

43. A method according to claim **41**, wherein each metal oxide particle comprises one or more materials independently selected from the group consisting of oxides of antimony and oxides of metals present within Group III or Group IV of the Periodic Table.

44. A method according to claim **43**, wherein each metal oxide particle comprises one or more materials independently selected from the group consisting of tin oxide, titania, antimony oxide, silica, alumina, silicalite and fumed silica.

45. A method according to claim **41**, wherein the solution comprises 0.2 to 25 weight percent of metal oxide particles with a primary particle size of less than 1000 Å.

46. A method according to claim **41**, wherein the solution further comprises extended polymers of a substantially hydrolyzed metal alkoxide linked to the metal oxide particles, wherein the weight ratio of metal oxide particles to the substantially hydrolyzed metal alkoxide ranges from 1 to 1000.

47. A method according to claim **46**, wherein the solution comprises 20 μmole to 2000 μmole of metal alkoxide per gram of metal oxide particles.

48. A method according to claim **46**, wherein the solution comprises from 60 μmole to 240 μmole of metal alkoxide per gram of metal oxide particles.

49. A method according to claim **46**, wherein the substantially hydrolyzed metal alkoxide is substantially hydrolyzed tetraethoxysilane.

50. A method according to claim **46**, wherein the solution has a pH ranging from 4 to 5.

51. A method according to claim **46**, wherein the volatile liquid is 70 to 90 volume percent ethanol, with the balance water.

52. A method according to claim **46**, wherein the solution is aged for greater than one day at 4° C. prior to application to the substrate.

53. A method according to claim **41**, wherein the layer is cured at at least 120° C. for 15 minutes.

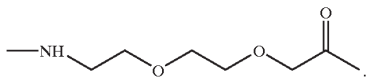
54. A method according to claim **41**, wherein at least one compound is attached to the substrate by a linker.

55. A method according to claim **54**, wherein the linker is an organoalkoxysilane molecule attached to the porous coating via a siloxane bond.

56. A method according to claim 55, wherein the linker is 3-amino-propyltriethoxysilane or bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane.

57. A method according to claim 54, wherein the linker comprises a photocleavable moiety, an enzyme cleavable moiety, an acid labile moiety or a base labile moiety.

58. A method according to claim 53, wherein the linker comprises the formula:



59. A method according to claim 42, wherein following evaporation of the volatile liquid and before the step of curing, the method comprises the steps of: (i) applying a fortifying solution comprising an aged sol of hydrolyzed tetraethoxysilane in a volatile solvent to the layer; and (ii) evaporating the volatile solvent to yield a fortifying layer wherein the weight ratio of metal oxide particles to the hydrolyzed tetraethoxysilane ranges from 1 to 1000.

60. A method according to claim 59, wherein the fortifying solution is an aged sol comprising 0.5 volume percent tetraethoxysilane, 0.15 volume percent water and 0.1 mM nitric acid, with the balance ethanol.

61. A method according to claim 41, wherein the substrate comprises an adhesive layer.

62. A method according to claim 61, wherein the adhesive layer comprises a polymer of hydrolyzed tetraethoxysilane.

63. A method according to claim 62, wherein the adhesive layer is generated by:

(i) applying onto the substrate a substantially uniform layer of an adhesive solution comprising an aged sol of hydrolyzed tetraethoxysilane in a volatile solvent; and

(ii) evaporating the volatile solvent from the layer and curing the layer to deposit a 0.002 to 2 micron thick adhesive layer of polymer of hydrolyzed tetraethoxysilane.

64. A method according to claim 63, wherein the adhesive solution is an aged sol comprising 1.5 volume percent tetraethoxysilane, 0.45 volume percent water, and 0.3 mM nitric acid with the balance ethanol.

65. A method according to claim 63, wherein the volatile solvent is evaporated at room temperature.

66. A method according to claim 63, wherein the adhesive layer is cured at at least 120° C. for at least 15 minutes.

67. A method according to claim 41, wherein at least 10^4 different compounds are attached to the porous coating at known discrete full thickness volumes of the porous coating.

68. A method according to claim 41, wherein each of the known discrete full thickness volumes occupies an area on the substrate of less than about 10,000 μm^2.

69. A method according to claim 41, wherein the compounds are attached to discrete known regions by a process comprising the steps of:

(i) attaching first molecules to the porous coating;

(ii) covering the first molecules with a layer of photoresist;

(iii) irradiating the photoresist such that photoresist is removed from first molecules in a first region, but not from first molecules in a second region;

(iv) contacting first molecules from which photoresist has been removed with a first reagent, forming second molecules attached the porous coating; and

(v) removing remaining photoresist.

70. A method according to claim 69, wherein the photoresist is a positive photoresist comprising a polyamide.

71. A coated article comprising a substrate having at least two discrete known regions with continuous porous coatings, wherein each coating has a substantially uniform thickness and comprises a gelled network of particles, and wherein each porous coating has at least one compound attached thereto, and wherein a different compound is attached to each of the porous coatings.

72. A coated article according to claim 71, wherein each particle comprises one or more materials independently selected from the group consisting of carbon, activated carbon, fluorinated carbon, styrenedivinylbenzene copolymers, polystyrene, zeolites, oxides of antimony and oxides of metals present within Group III and Group IV of the Periodic Table.

73. A coated article according to claim 72, wherein each particle comprises one or more materials independently selected from the group consisting of alumina, silica, silicalite, fumed silica, oxides of tin and titania.

74. A coated article according to claim 72, wherein the particles are substantially spherical silica particles.

75. A coated article according to claim 71, wherein the particles have a primary particle size of less than 1000 Å.

76. A coated article according to claim 71, wherein the continuous gelled network of particles further comprises a polymer of a substantially hydrolyzed metal alkoxide.

77. A coated article according to claim 76, wherein the substantially hydrolyzed metal alkoxide is substantially hydrolyzed tetraethyoxysilane.

78. A coated article according to claim 71, wherein the substrate comprises an adhesive layer.

79. A coated article according to claim 78, wherein the adhesive layer comprises polymers of hydrolyzed tetraethoxysilane.

80. A coated article according to claim 71, wherein the substrate has more than 10^4 separate porous coatings with attached compounds.

81. A coated article according to claim 71, wherein at least one compound is attached to the porous coatings via a linker.

82. A coated article according to claim 81, wherein the linker is an organoalkoxysilane molecule attached to the porous coatings via siloxane bonds.

83. A coated article according to claim 81, wherein the linker comprises a photocleavable moiety or an enzyme cleavable moiety.

84. A coated article according to claim 81, wherein the linker comprises an acid labile moiety or a base labile moiety.

85. A coated article according to claim 71, wherein the attached compounds are selected from the group consisting of nucleobase polymers, peptides and enalaprilat analogues.

86. A coated article according to claim 71, wherein each of the porous coatings further comprises a fortifying layer of a polymer of hydrolyzed tetraethoxysilane.

87. A coated article according to claim 71, wherein the average pore size of each of the separate porous coatings substantially approximates the particle size.

88. A method of making a coated article comprising a substrate and at least two separate porous coatings, comprising the steps of:

(a) applying to a substrate a substantially uniform layer of a solution comprising metal oxide particles dispersed in a volatile liquid;

(b) evaporating the volatile liquid from the layer, forming a gelled network of metal oxide particles on the substrate, wherein the gelled network forms a porous coating ranging from 0.05 to 25 microns thick;

(c) covering the porous coating with a layer of photoresist comprising a base soluble component;

(d) irradiating the photoresist, such that a first region of photoresist is rendered substantially removable with an aqueous alkaline developer, and such that a second region is not so removable;

(e) contacting at least the first region with an aqueous alkaline developer to remove at least the first region of photoresist and porous coating underlying the first region, without substantially removing the second region of photoresist or porous coating underlying the second region;

(f) removing remaining photoresist with an organic solvent, resulting in separate porous coatings on discrete regions of the substrate; and

(g) attaching one or more compounds to each of the separate porous coatings, and therefrom generating a substrate having at least two separate porous coatings, wherein each coating has a substantially uniform thickness and comprises a continuous gelled network of metal oxide particles and polymers of a hydrolyzed metal alkoxide, and wherein a different compound is attached to each of the porous coatings.

89. A method according to claim **88**, wherein each metal oxide particle comprises one or more materials independently selected from the group consisting of oxides of antimony and oxides of metals present within Group III or Group IV of the Periodic Table.

90. A method according to claim **89**, wherein each metal oxide particle comprises one or more materials independently selected from the group consisting of tin oxide, titania, antimony oxide, silica, alumina, silicalite and fumed silica.

91. A method according to claim **88**, wherein the metal oxide particles are substantially spherical particles of silica.

92. A method according to claim **88**, wherein at least one compound is attached via a linker.

93. A method according to claim **88**, wherein the solution comprises 0.2 to 25 weight percent of metal oxide particles with a primary particle size of less than 500 Å.

94. A method according to claim **88**, wherein the solution comprises 20 μmole to 2000 μmole of metal alkoxide per gram of metal oxide particles.

95. A method according to claim **88**, wherein the solution comprises 60 μmole to 240 μmole of metal alkoxide per gram of metal oxide particles.

96. A method according to claim **88**, wherein the substantially hydrolyzed metal alkoxide is substantially hydrolyzed tetraethoxysilane.

97. A method according to claim **88**, wherein the solution has a pH ranging from 4 to 5.

98. A method according to claim **88**, wherein the volatile liquid is 70 to 90 volume percent ethanol, with the balance water.

99. A method according to claim **88**, wherein the solution is aged for greater than one day at 4° C.

100. A method according to claim **88**, wherein the volatile liquid is evaporated at room temperature.

101. A method according to claim **88**, wherein the photoresist comprises a diazoquinone.

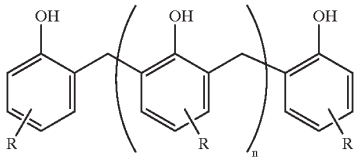
102. A method according to claim **88**, wherein the radiation is selected from the group consisting of coherent, incoherent, x-ray, deep ultraviolet, mid ultraviolet, near ultraviolet, visible and infrared light.

103. A method according to claim **88**, wherein the step of irradiating comprises:

(i) placing a mask between a light source and the layer of photoresist, wherein the mask comprises first regions that transmit at least one selected wavelength of light and second regions that do not substantially transmit the selected wavelength of light.

(ii) irradiating the mask with a light source emitting the selected wavelength, such that light is transmitted to at least a first region of the layer of photoresist and is not substantially transmitted to at least the second region of the layer of photoresist.

104. A method according to claim **88**, wherein the base soluble component is a phenolic polymer having the formula:



wherein R is hydrogen or an alkyl group, and n is an average ranging from 0 to 13.

105. A method according to claim **88**, wherein the aqueous alkaline developer comprises a base selected from the group consisting of potassium hydroxide, sodium hydroxide and tetramethyl ammonium hydroxide.

106. A method according to claim **88**, wherein the organic solvent is acetone.

107. A method according to claim **88**, wherein each separate porous coating is less then 25 microns thick.

108. A method according to claim **88**, wherein at least one compound is attached via a linker.

109. A method according to claim **88**, wherein prior to the step of attaching one or more compounds, the separate porous coatings are cured at a temperature and for time sufficient to increase the porous coating strength.

110. A method according to claim **88**, wherein following removal of remaining photoresist and before the step attaching one or more compounds, the method comprises the steps of: (i) applying a fortifying solution comprising an aged sol of hydrolyzed tetraethoxysilane in a volatile solvent to the layer of aged solution; and (ii) evaporating the volatile solvent to yield a fortifying layer wherein the weight ratio of metal oxide particles to the hydrolyzed tetraethoxysilane ranges from 1 to 1000.

111. A method according to claim **110**, wherein the fortifying solution is an aged sol comprising 0.5 volume percent tetraethoxysilane, 0.15 volume percent water, and 0.1 mM nitric acid with the balance ethanol.

112. A method according to claim **88**, wherein the substrate comprises an adhesive layer of a polymer of hydrolyzed tetraethoxysilane.

113. A method according to claim **112**, wherein the adhesive layer is generated by:

(i) applying onto the substrate a substantially uniform layer of an adhesive solution comprising an aged sol of hydrolyzed tetraethoxysilane in a volatile solvent; and

(ii) evaporating the volatile solvent from the layer and curing the layer to deposit a 0.002 to 2 micron thick adhesive layer of polymer of hydrolyzed tetraethoxysilane.

114. A method according to claim **113**, wherein the adhesive solution is an aged sol comprising 1.5 volume percent tetraethoxysilane, 0.45 volume percent water and 0.3 mM nitric acid, with the balance ethanol.

115. A method according to claim **113**, wherein the volatile solvent is evaporated at room temperature.

116. A method according to claim 113, wherein the adhesive layer is cured at at least 120° C. for at least 15 minutes.

117. A method according to claim 88, wherein the substrate has more than 10^4 separate porous coatings in known regions, each of the porous coatings with different attached compounds.

118. A method according to claim 88, wherein each of the separate porous coatings occupies an area on the substrate of less than about 10,000 μm^2.

119. A method according to claim 88, wherein the photoresist is a positive photoresist.

120. A method according to claim 88, wherein the photoresist is a negative photoresist.

121. A method according to claim 88, wherein the compounds are attached to discrete known regions by a process comprising the steps of:

(i) attaching first molecules to the separate porous coatings;

(ii) covering the first molecules by a layer of second photoresist;

(iii) irradiating at least a portion of the second photoresist, such that second photoresist is removed from the first molecules in the first region;

(iv) contacting first molecules from which photoresist has been removed with a first reagent, forming second molecules attached to the separate porous coatings; and

(v) removing remaining second photoresist.

122. A method according to claim 121, wherein the second photoresist comprises a polyamide.

123. A method according to claim 121, wherein the second photoresist is a positive photoresist.

124. A method according to claim 121, wherein the second photoresist is a negative photoresist.

125. A method of identifying at least one compound that specifically binds a receptor, the method comprising the sequential steps of

(a) providing a coated article according to claim 1 or claim 72;

(b) contacting said coated article with a receptor; and

(c) determining whether one or more of the compounds attached to the porous coating specifically bind to the receptor.

126. A method according to claim 125, wherein each compound attached to the porous coating is independently selected from the group consisting of nucleobase polymers and peptides.

127. A method according to claim 126, wherein the compounds are antisense nucleic acid molecules.

128. A method according to claim 125, wherein the receptor is a nucleobase polymer, enzyme, cell receptor or antibody.

129. A method according to claim 125, wherein the receptor further comprises a detectable marker and wherein step (b) comprises detecting a location of the marker on the porous coating.

130. A method according to claim 129, wherein the marker is a radioactive marker or a fluorescent marker.

131. A method for identifying at least one compound that specifically binds a receptor, the method comprising the steps of:

(a) providing a coated article according to claim 1 or claim 71;

(b) simultaneously or in either order:

(i) detaching one or more compounds from said coated article; and

(ii) contacting the detached compound(s) with a receptor, and

(c) determining whether the compound(s) specifically bind to the receptor.

132. A method according to claim 131, wherein the compounds are attached to the substrate via a linker.

133. A method according to claim 132, wherein the linker comprises a photocleavable moiety, and wherein the compound(s) are detached from the substrate by irradiation with light.

134. A method according to claim 133, wherein the light is selected from the group consisting of ultraviolet, visible and infrared light.

135. A method according to claim 132, wherein the linker comprises a recognition site that is cleaved by an enzyme, and wherein the compound(s) are detached from the substrate by contact with an enzyme.

136. A method according to claim 132, wherein the linker comprises an acid labile moiety or a base labile moiety, and wherein the compound(s) are detached from the substrate by contact with an acidic or basic chemical.

137. A method according to claim 136, wherein the chemical is selected from the group consisting of liquid trifluoroacetic acid, gaseous trifluoro acetic acid, liquid ammonia and gaseous ammonia.

138. A method according to claim 131, wherein the receptor is an enzyme.

139. A method according to claim 138, wherein the enzyme is angiotensin converting enzyme.

140. A method according to claim 131, wherein step (c) further comprises:

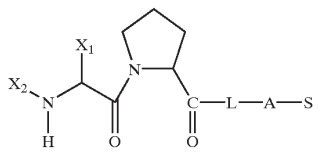
(i) contacting the receptor with an indicator compound having a detectable property in the presence of receptor bound to a compound; and

(ii) determining the presence or absence of the detectable property.

141. A method according to claim 140, wherein the detectable property is selected from the group consisting of color, light absorbance, light transmission, fluorescence, fluorescence resonance energy transfer, fluorescence polarization, phosphorescence, catalytic activity, molecular weight, charge, density, melting point, chromatographic mobility, turbidity, electrophoretic mobility, mass spectrum, ultraviolet spectrum, infrared spectrum, nuclear magnetic resonance spectrum, elemental composition, and x-ray diffraction.

142. A method according to claim 141, wherein the indicator compound is furylacryloylphenyalanylglycylglycine and wherein the receptor is angiotensin converting enzyme.

143. A method according to claim 131, wherein the coated article comprises enalaprilat analogues of the formula:



wherein S is the porous coating, A is aminopropyltriethoxysilane, L is a divalent linker molecule, X_1 is a monovalent organic group or hydrogen, and X_2 is a monovalent organic group or hydrogen.

144. A method for isolating a target receptor, comprising the steps of:

(a) providing a coated article according to claim 1 or claim 71;

(b) contacting said coated article with a composition comprising a target receptor, wherein at least one attached compound binds to the target receptor;

(c) removing unbound components of the composition from the array; and

(d) separating the target receptor from the coated article, and therefrom isolating the target receptor.

145. A coated article according to claim **1** or claim **71**, wherein at least 5% of the attached compounds comprise a target receptor modifying group that labels, reconforms, cleaves, covalently binds or intercalates into a bound target receptor.

146. A method for modifying a receptor, comprising the steps of:

(a) providing a coated article according to claim **145**; and

(b) contacting said coated article with a composition comprising a target receptor.

147. A method for hybridizing an antisense molecule to a target nucleic acid molecule, comprising the steps of:

(a) providing a coated article according to claim **1** or claim **71**;

(b) contacting said coated article with a composition comprising a target nucleic acid molecule, wherein the attached compounds are antisense molecules; and

(c) detaching one or more compounds from the array, and thereby hybridizing an antisense molecule to the target nucleic acid molecule.

148. A method for hybridizing an antisense molecule to a target nucleic acid molecule, comprising the steps of:

(a) providing a coated article according to claim **1** or claim **71**;

(b) detaching one or more compounds from said coated article, wherein the attached compounds are antisense molecules; and

(c) contacting the compound(s) with a composition comprising a target nucleic acid molecule, and thereby hybridizing an antisense molecule to the target nucleic acid molecule.

149. A coated article according to claim **1** or claim **71**, wherein the attached compounds are nucleobase polymers, wherein the nucleobase polymers comprise at least one set of 2 to 10 different probes of identical length, wherein:

(a) one probe is completely complementary to a 4 to 40 nucleotide portion of a reference sequence first set that is exactly complementary to a reference sequence and comprises nucleobase polymers that completely span the reference sequence and, relative to the reference sequence, overlap one another; and

(b) the remaining probe(s) of the set are each identical to the completely complementary probe except that each contains one nucleobase substitution relative to the completely complementary probe, wherein each substitution is at the same position relative to the reference sequence.

150. A coated article comprising a substrate having a plurality of continuous porous coatings thereon of substantially uniform thickness, wherein each of the porous coatings comprises a continuous gelled network of metal oxide particles and polymers of hydrolyzed metal alkoxide, and wherein each of the porous coatings has a surface area measuring greater than 50 meters^2/g.

151. A coated article according to claim **150**, wherein the metal alkoxide is tetraethoxysilane.

152. A coated article comprising a substrate having at least five separate distinct porous coatings per square centimeter, wherein each coating is continuous and has a substantially uniform thickness and comprises a continuous gelled network of particles, and wherein each of the separate porous coatings occupies an area on the substrate of less than about 1,000,000 μm^2.

153. A coated article according to claim **152**, wherein the substrate has at least 100 separate distinct porous coatings per square centimeter.

154. A coated article comprising a substrate having at least two discrete known regions with continuous porous coatings, wherein each coating has a substantially uniform thickness and comprises a gelled network of particles, and wherein each porous coating has at least one compound attached thereto, and wherein each of the separate porous coating occupies an area on the substrate of less than about 10,000 μm^2.

155. A coated article comprising a substrate having at least two discrete known regions with continuous porous coatings, wherein each coating has a substantially uniform thickness and comprises a gelled network of particles, and wherein each porous coating has at least one compound attached thereto, and wherein the porous coatings have a surface area measuring greater than 50 meters ^{2}/g.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

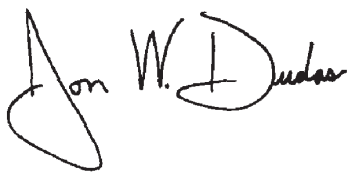
PATENT NO. : 6,951,682 B1
DATED : October 4, 2005
INVENTOR(S) : John A. Zebala

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 89,
Line 49, the period "." within the numeral "1,000.000" should read as a comma -- , --.

Signed and Sealed this

Twenty-ninth Day of November, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

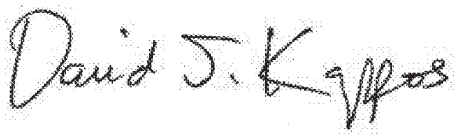
PATENT NO.	: 6,951,682 B1
APPLICATION NO.	: 09/332815
DATED	: October 4, 2005
INVENTOR(S)	: John A. Zebala

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, Line 9 please insert a Government Rights Statement as shown below:

--This invention was made with government support under Grant Number DAMD17-96-1-6120 awarded by the U.S. Army Medical Research and Material Command. The government has certain rights to this invention.--

Signed and Sealed this
Eighth Day of January, 2013



David J. Kappos
Director of the United States Patent and Trademark Office

(12) **EX PARTE REEXAMINATION CERTIFICATE** (7586th)

United States Patent
Zebala

(10) **Number: US 6,951,682 C1**
(45) **Certificate Issued: Jun. 29, 2010**

(54) **POROUS COATINGS BEARING LIGAND ARRAYS AND USE THEREOF**

(75) Inventor: **John A. Zebala**, Redmond, WA (US)

(73) Assignee: **Syntrix Biosystems, Inc.**, Auburn, WA (US)

Reexamination Request:
No. 90/011,998, Jun. 11, 2008

Reexamination Certificate for:

Patent No.:	**6,951,682**
Issued:	**Oct. 4, 2005**
Appl. No.:	**09/332,815**
Filed:	**Sep. 17, 1999**

Certificate of Correction issued Nov. 29, 2005.

Related U.S. Application Data

(60) Provisional application No. 60/110,529, filed on Dec. 1, 1998.

(51) **Int. Cl.**

C03C 17/00	(2006.01)
C07B 61/00	(2006.01)
C12Q 1/68	(2006.01)
C12Q 1/37	(2006.01)
G01N 33/552	(2006.01)
G01N 33/551	(2006.01)
B01J 19/00	(2006.01)
C07H 21/00	(2006.01)
C07K 5/02	(2006.01)
C07K 5/00	(2006.01)

(52) **U.S. Cl.** **428/312.2**; 428/312.8; 428/315.7; 428/317.1; 435/4; 435/7.1; 435/7.7; 435/7.71; 435/7.72; 435/7.8; 435/7.9; 436/518; 436/519; 436/523

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,601,123	A	6/1952	Moulton
3,910,851	A	10/1975	Messing
4,816,333	A	3/1989	Lange et al.
5,120,600	A	6/1992	Pirrung et al.
5,224,972	A	7/1993	Frye et al.
5,268,198	A	12/1993	Yamasaki et al.
5,519,088	A	5/1996	Itoh et al.
5,589,396	A	12/1996	Frye et al.
5,624,875	A	4/1997	Nakanishi et al.
5,624,998	A	4/1997	Itoh et al.
5,686,602	A	11/1997	Farooq et al.
5,879,881	A	3/1999	Rubenstein
5,900,481	A	5/1999	Lough et al.
6,023,540	A	2/2000	Walt et al.
6,951,682	B1	10/2005	Zebala

FOREIGN PATENT DOCUMENTS

JP	59001777	1/1984
JP	5254819	10/1993
WO	WO90/05910	5/1990
WO	WO94/14088	6/1994
WO	WO 97/16569	5/1997
WO	WO 97/39151	10/1997
WO	WO 97/40385	10/1997
WO	WO 99/02266	1/1999
WO	WO 00/33078	6/2000

OTHER PUBLICATIONS

Beattie et al. "Advances in Genosensor Research" Clin. Chem. 41(5):700–706, 1995.

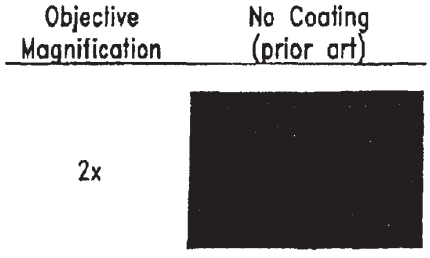
Cathro et al. "Silica Low–Reflection Coatings for Collector Covers By a Dip–Coating Process" Solar Energy 32 (5):573–579, 1984.

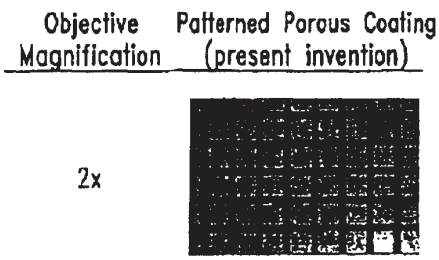
(Continued)

Primary Examiner—Stephen J Stein

(57) **ABSTRACT**

Articles comprising substantially uniform porous coatings, which may be photopatterned, are provided. The use of such porous coatings increases the surface density of attached compounds within, for example, ligand arrays prepared by methods such as regionally selective solid-phase chemical synthesis. Arrays prepared using the porous coatings may be used within a variety of diagnostic and drug discovery assays.





OTHER PUBLICATIONS

Chu et al. "Glycidoxypropyltrimethoxysilane Modified Colloifal Silica Coatings" Mat. Res. Soc. Symp. Proc. 435:221–225, 1996.

Daniels et al. "Use of an Organosilane Coupling Agent in Colloidal Silica Coatings" Mat. Res. Soc. Symp. Proc. 435:215–220, 1996.

Frye et al. "Sol–Gel Coatings on Acoustic Wave Devices: Thin Film Characterization and Chemical Sensor Development" Mat. Res. Soc. Symp. Proc. 180:583–593, 1990.

Guschin et al. "Manual Manufacturing of Oligonucleotide, DNA and Protein Microchips" Anal. Biochem. 250 (2):203–211, 1997.

Pu et al. "Photobinding of Colloidal Particles by Means of Surface Modification. IV. Negative and Positive Working Imaging by Control of Substrate Surface Energy" J. Imaging Sci. 33(5):177–183, 1989.

Yershov et al. "DNA Analysis and Diagnostics on Oligonucleotide Microchips" Proc. Natl. Acad. Sci. USA 93:4913–4918, 1996.

EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims **1-7**, **9**, **10**, **15**, **16**, **19-23**, **28-33**, **37-40**, **154** and **155** is confirmed.

Claims **71** and **125** are determined to be patentable as amended.

Claims **72-75**, **78**, **80**, **81**, **83-85**, **87** and **126-130**, dependent on an amended claim, are determined to be patentable.

Claims **8**, **11-14**, **17**, **18**, **24-27**, **34-36**, **41-70**, **76**, **77**, **79**, **82**, **86**, **88-124** and **131-153** were not reexamined.

71. A coated article comprising a substrate having at least two discrete known regions with continuous porous coatings, wherein each *porous* coating has a substantially uniform thickness and comprises a gelled network of particles, and wherein each porous coating has at least one compound attached thereto, and wherein a different compound is attached to each of the porous coatings.

125. A method of identifying at least one compound that specifically binds a receptor, the method comprising the sequential steps of (a) providing a coated article according to claim **1** or claim **[72]** *71*; (b) contacting said coated article with a receptor; and (c) determining whether one or more of the compounds attached to the porous coating specifically bind to the receptor.

* * * * *

CERTIFICATE OF SERVICE AND FILING

I certify that I electronically filed the foregoing document using the Court's CM/ECF filing system. Counsel was served via CM/ECF on March 12, 2014.

Mr. Daniel E. O'Toole
Clerk of Court
United States Court of Appeals for the Federal Circuit
717 Madison Place, N.W.
Room 401
Washington, DC 20439

William F. Lee — *Attorneys for Appellee*
Louis W. Tompros
Wilmer Cutler Pickering Hale and Dorr LLP
60 State Street
Boston, MA 02109

Alan D. Albright, Esq.
Bracewell & Giuliani, LLP
111 Congress Avenue
Suite 2300
Austin, TX 78701

Derek Tod Gilliland
Christian John Hurt
Nix Patterson & Roach LLP
205 Linda Drive
Daingerfield, TX 75638

Michael R. Samardzija, Esq., -
Bracewell & Giuliani, LLP
711 Louisiana Street
S. Tower Pennzoil Place, Suite 2300
Houston, TX 77002

/s/ Craig E. Countryman
Craig E. Countryman

CERTIFICATE OF COMPLIANCE

The undersigned attorney certifies that the opening brief for Appellant Illumina, Inc. complies with the type-volume limitation set forth in Fed. R. App. P. 32(a)(7)(B). The relevant portions of the brief, including all footnotes, contain 11,634 words as determined by Microsoft Word.

Dated: March 12, 2014

/s/ Craig E. Countryman
Craig E. Countryman